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Some Spermatological Aspects of Channel Catfish, Ictalurus Punctatus (Rafinesque)
Dissertation directed by Professor James W. Avault, Jr.
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ABSTRACT

Males of a wild stock of channel catfish, Ictalurus punctatus (Rafinesque) from Lake des Allemands, Louisiana were compared with males belonging to a domestic stock from Yazoo City, Mississippi for several aspects relating to their reproductive physiology.

The study started in November 1971 and continued through April 1972. Each month four fish of each stock, comprising two 2-year-olds and two 3-year-olds were examined.

The following body measurements were taken : weight, total length, width and circumference of the head. The body weight varied between 142 and 2,050 g with an average of 687 g. The 3-year-old fish were considerably larger than the 2-year-olds, and the domestic stock outgrew the wild one.

In the testes two distinct parts could be recognized, an anterior spermatogenic one which is white

and consists of large lobules of very soft tissue, and a glandular one, pinkish in color and made up of very fine lobules of harder tissue. The spermatogenic part was devided in three consecutive areas and the variables measured in each one. Little variation among these parts existed. The spermatogenic part averaged 1.14 g, the glandular only 0.56 g. In early spring both parts increased appreciably in weight and volume.

In both stocks a case of azoospermia occurred, both were 3-year-old fish with very pronounced male secondary sexual characteristics and well developed testes that appeared normal in all respects but did not contain spermatozoa.

The spermatozoal motility score was usually high with most cells in progressive motion and a few vibrating in loco. The percent live spermatozoa was determined using a differential eosin-nigrosin stain. On the average 73.2% were alive.

Spermatozoal morphology, including dimensions, aberrations and ultrastructures were studied. The channel catfish spermatozoon had a rounded head 2.3 µm long and 2.4 µm wide, a collar-like midpiece containing diffuse mitochondria and 1.6 µm in length and 3.1 µm in width, and a 94.9 µm long flagellum.

Biflagellar spermatozoa each arising from an

individual centriolar complex occurred in every fish at the average rate of 4.7%. An apparently split flagellum singly implanted in the head but separated longitudinally in some areas of the tail was encountered in 9.4% of the spermatozoa.

The mean gonadal sperm concentration was approximately 5.5×10^9 spermatozoa/g wet testicular tissue. Values increased from February on reaching 7.4×10^9 in April. Both stocks had similar concentrations but 3-year-olds produced relatively more than 2-year-olds. The gonosomatic index was only 0.22% on the average.

In a separate experiment the influence of 0, 2, 5, and 8‰ saline solutions at 24, 27, and 30 C on spermatozoal motility in vitro was tested during 720 minutes at 20 minute intervals. Salinity seemed to have more effect on motility than temperature. In 0‰ spermatozoa were immotile after 5 minutes, regardless of the temperature level. The 5‰ salinity seemed to be the optimal one of those tested and maintained vigorous progressive motion of the spermatozoa for at least 360 minutes.

For most of the variables little difference existed between the wild and domestic stock but some size and/or age effects were noticed.

SOME SPERMATOLOGICAL ASPECTS OF CHANNEL CATFISH, *ICTALURUS PUNCTATUS* (RAFINESQUE)

A Dissertation

Submitted to the Graduate Faculty of the
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in

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For most of the variables little difference existed between the wild and domestic stock but some size and/or age effects were noticed.

INTRODUCTION

Commercial pond culture of channel catfish, Ictalurus punctatus (Rafinesque) is mainly concentrated in the Mississippi River Delta region. Approximately 30,300 ha are presently under catfish production in the United States (personal communication Mayo Martin, Fish Farming Experimental Station, Stuttgart, Arkansas) and acreage is increasing. Of the total United States cultured yield, 80% is produced by only three states : Arkansas, Mississippi, and Louisiana (Anonymous, 1970). Annual profit for the catfish farmer can exceed $ 495/ha (Avault et al., 1969).

Even though the channel catfish is successfully grown on a commercial basis, various aspects of its biology remain rather obscure. The most important area where knowledge is lacking concerns the reproductive physiology. Research is in progress to genetically improve channel catfish stocks through selective breeding and thus increase their commercial value. A clear insight in the physiological aspects of reproduction is only one of the prerequisites to achieve this goal.

Cryogenic preservation of semen and artificial insemination of farm animals have resulted in tremendous

improvements of breeds (Johansson and Rendel, 1968), and show great potential in fish culture. The first step, however, enabling us to apply these sophisticated methods for storing male gametes and subsequently artificially fertilize eggs, is to gain a detailed knowledge of the spermatogenic cycle and spermatozoal physiology.

At present virtually no information pertaining to channel catfish is available on these subjects. The morphology of its spermatozoa has not been described, nor are there any data on sperm motility, viability, and gonadal sperm concentration.

In the laboratory, channel catfish have been successfully grown from the egg in water with a salinity up to $8\,^{\circ}/_{oo}$ (Allen and Avault, 1969). Perry and Avault (1969) cultured channel catfish in brackish water ponds with salinities ranging from 2 to 11 $^{\circ}/_{oo}$ but did not obtain spawning. Channel catfish is one of many fish species under consideration for mariculture and it is imperative to examine the influence of temperature and salinity on the spermatozoa of this fresh water species.

The objectives of this study were to compare a domestic versus a wild stock of male channel catfish for the following characteristics :

1) body and testes properties
2) spermatozoal motility and viability
3) spermatozoal morphology, including dimensions, occurrence and type of aberrations, and ultrastructures

4) gonadal sperm concentration

5) influence of temperature and salinity on spermatozoal motility

This study extends the knowledge concerning the reproductive physiology of male channel catfish and hopefully represents a basis of catfish spermatology. Thus it may eventually lead to man's control over and preservation of male catfish gametes, which are necessary to exploit the available potential of superior males to its fullest degree.

MATERIALS AND METHODS

Experimental Fish

The wild channel catfish fry used in the study were obtained from Lake des Allemands, Louisiana and the domesticated hatchery fry from Yazoo City, Mississippi through the Thompson-Anderson Enterprises. The ancestors of the Mississippi fish were originally obtained from the Yazoo River, Mississippi, but had been cultured in ponds for more than 10 years.

Fry from both areas were stocked separately in adjacent, 0.04 ha earthen ponds, at 500 fry per pond (12,500/ha). A first stocking was carried out in late May and early June, 1969. At the beginning of the study, conducted from November 1971 through April 1972, these fish were $2\frac{1}{2}$ years old ; they were 3 years at the end of the study and will be referred to as 3-year-olds. A second batch of fry from each location was stocked separately in the summer of 1970. These fish were $1\frac{1}{2}$ years old at the beginning of the study, and 2 years at the end ; and they will be referred to as 2-year-olds.

During the growing-out period, fry were fed a complete sinking pelleted commercial trout feed (Purina Trout Chow)[1] daily and ad libitum. Once the fingerlings reached approximately 10 cm, a floating pelleted commercial catfish feed (Purina Catfish Chow FR)[1] was used. Forage fishes in the ponds consisted of Tilapia aurea (Steindachner) and gizzard shad, Dorosoma cepedianum (Lesueur).

Each month two male channel catfish from each stock and age group were seined from the ponds for the experiments which lasted from November 1971 through April 1972. These eight male fish were maintained outdoors in aerated tanks, adjacent to the laboratory until studied. Thus, during the project, 24 male fish from each stock, comprising 12 of each age group within the stock, were sacrificed and examined for the characteristics outlined below .

Body Measurements

The male channel catfish were stunned by electric shocking, and blotted dry. First observations were made on secondary sexual characteristics such as protrusility of the genital papilla and degree of dark pigmentation of the mandibular region. Then, the following body measurements were taken : body weight (g), total body length (mm),

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[1] The use of trade names does not imply endorsement of the commercial products.

width and circumference of the head (mm), immediately anterior to the pectoral spine.

Spermatozoal Motility

Following these measurements, a longitudinal incision was made ventrally from the genital papilla anteriorly to the pectoral girdle, thus exposing the testes. The testes have two distinct regions, a white spermatogenic one anteriorly, and a pinkish non-spermatogenic one toward the genital papilla. Sneed and Clemens (1963) assumed the posterior non-spermatogenic part to have a glandular function. In this study, this region of the catfish testes will therefore be referred to as the glandular part, even though its secretive activity is still hypothetical. The color, general morphology and texture of the testes were recorded.

Three small tissue samples, each approximately pin head size, were collected consecutively from the spermatogenic part of the testes, one anteriorly, one medially, and one posteriorly, as well as a tissue sample from the glandular part.

To release the spermatozoa, each tissue sample was teased apart in a drop of 0.65% sodium chloride (NaCl) solution, on a glass slide. In preliminary investigations this concentration of saline solution was proven most adequate to suspend spermatozoa without causing morphological distortions of the cells. The wet preparation was

then covered with a number 1 cover slip and examined under a phase contrast microscope at a magnification of 1,569 X.

The spermatozoal motility was visually assessed and according to the criteria in Table 1, established during preliminary observations, an appropriate score was assigned.

Table 1. Criteria to assess spermatozoal motility and corresponding motility score

Criteria	Motility score
All spermatozoa progressively motile with vigorous flagellar movements	5
Most spermatozoa progressively motile with vigorous flagellar movements, some spermatozoa exhibiting strong vibrations in loco	4
Most spermatozoa progressively motile, the others vibrating in loco	3
Most spermatozoa vibrating in loco, with a few exhibiting progressive motion	2
Most spermatozoa vibrating in loco, but very few progressively motile	1
Most spermatozoa immotile, with very few slightly vibrating and very few with progressive motion	0.75
Most spermatozoa immotile, with very few slightly vibrating and an occasional one in progressive motion	0.50
Most spermatozoa immotile with an occasional one slightly vibrating	0.25
All spermatozoa immotile	0

Percent Live Spermatozoa

Since immotile spermatozoa are not necessarily dead, the sole motility score is a biased estimator of the number of living gametes. To determine the percent of live spermatozoa, a differential eosin-nigrosin staining technique was used. The method was originally described by Blom (1950) for bovine spermatozoa, but proven suitable to distinguish live from dead fish spermatozoa by Fribourgh (1966). Only dead cells absorb the eosin and are stained red, while the living ones remain white against the violet-brownish background.

The eosin stain was made by dissolving 5 g eosin bluish in 95 g distilled water, and the nigrosin counter-stain by dissolving 10 g nigrosin in 90 g distilled water.

Three small tissue samples were obtained from the three areas (anterior, medial and posterior) of the spermatogenic part of the testes. The tissue was teased apart in a drop of 0.65% NaCl solution, on a glass slide. To this suspension a drop of the eosin solution was added and thoroughly mixed ; then two drops of the nigrosin counter-stain was stirred in the mixture. The preparation was smeared over three glass slides with a glass rod and dried over a gentle flame. The three slides were used to avoid bias due to patchy staining.

The slides were examined the same day, under oil

immersion, with a light microscope at a magnification of 970 X.

For each area of the testes, a total of 200 spermatozoa were examined and a differential count of live versus dead gametes recorded and converted to percent live spermatozoa.

Testes Measurements

After determining the percent live spermatozoa, the testes were carefully dissected out, and freed from all connective tissue and coagulated blood. They were rinsed in a 0.65% NaCl solution and blotted dry on soft tissue paper. The spermatogenic part was then separated from the glandular part. The left and right sides of each part were combined, and the following measurements taken on both the spermatogenic and glandular parts separately : weight (to the nearest 0.01 g), length (mm), volume (to the nearest 0.01 cc) and width (mm). Determination of the volume was carried out by water displacement by placing the testicular material in a 12 cc centrifuge tube and adding a 0.65% NaCl solution from a syringe graduated in 0.01 cc divisions.

After the testes measurements, the sequential procedures could be interrupted if necessary. The spermatogenic and glandular parts were then suspended separately in a 0.65% NaCl solution in a capped vial, and stored overnight in a refrigerator at 4 C.

Spermatozoal Morphology

Stains

In preliminary experiments the efficiency of three stains : rose Bengal, fast green, and pinacyanol chloride to color channel catfish spermatozoa were tested. Of the first stain, two solutions of different composition were tried. One consisted of 3 g powdered rose Bengal, 99 cc distilled water, and 1 cc of 40% formaldehyde, while in the other, the distilled water was replaced by 99 cc of a 0.65% NaCl solution, the other components remaining the same.

For both rose Bengal stains the following procedures were used : spermatozoa were teased from fresh testicular tissue and suspended in a drop of a 0.65% NaCl solution on a glass slide. A smear was made and the saline solution evaporated by air drying. A drop of rose Bengal was added and after 5 seconds, the slide was gently washed in running tap water to remove the excess stain. The preparation was blotted dry and examined under both light and phase contrast microscopes at magnifications of respectively 970 and 1,569 X.

The fast green stain was used as a $1^{o}/_{oo}$ aqueous solution. Spermatozoa were teased from fresh testicular tissue and a drop of stain added. After gentle mixing of the preparation it was covered with a cover slip and viewed in the same manner as previously described.

The third stain tested consisted of 250 mg pinacyanol chloride dissolved in 100 cc of 70% ethyl alcohol (Cruea et al., 1969). Spermatozoa teased from fresh testicular tissue were suspended in a drop of a 0.65% NaCl solution on a glass slide and smeared out. The mixture was air dried and immersed in the pinacyanol chloride solution for 10 seconds. After rinsing in running tap water, the slide was blotted dry and examined in the same way as the other preparations.

Dimensions

The morphology of the channel catfish spermatozoa was studied separately in the anterior, medial and posterior areas of the spermatogenic testes part. A tissue sample from the appropriate region was teased apart in a drop of 0.65%NaCl on a glass slide, and covered with a number 1 cover slip. The sample was examined under oil immersion with a phase contrast microscope at a magnification of 1,569 X. The spermatozoal dimensions were determined to the nearest 0.8 μm with the ocular micrometer.

During the first 3 months of the study (November 1971 - January 1972), 20 spermatozoa were measured in each testicular area. Statistical analysis of these data revealed that, due to the small variation in the measurements, the number of observations could be reduced to five without losing accuracy. For the following 3 months of the study (February 1972 - April 1972) the number of observations per area was therefore reduced to five instead

of 20.

The following spermatozoal measurements were taken in micrometers : total length of the head and midpiece, length of the head, length of the midpiece (these three measurements were made along the longitudinal axis), width of the head, width of the midpiece (along the axis perpendicular to the longitudinal), and length of the flagellum.

Aberrations

After determining their dimensions, a total of 200 spermatozoa were examined for each of the three testicular areas. A differential count of the morphologically normal spermatozoa and those with aberrations was carried out. Only morphological aberrations pertaining to the flagellum were observed. Distinction was mede between biflagellar gametes and sperm cells with a flagellum that appeared double or split at some regions, and as a single flagellum in other regions of the tail. It is possible that the apparently split flagellum consisted in fact of two flagella overlapping each other at some parts of the tail.

The number of spermatozoa recorded in the differential count as either normal, biflagellar or with a split flagellum was then divided by two to give the respective percentiles.

Ultrastructure

The ultrastructure of channel catfish spermatozoa was investigated by electron microscopy.

In preliminary experiments, negative staining and carbon replication gave unsatisfactory results, but thin sectionning was successful.

Tissue from the spermatogenic part of the testes was macerated and fixed in a 3% gluteraldehyde, $CHO(CH_2)_3CHO$ solution at 4 C for 2 hours, and left overnight in Sorensen's phosphate buffer with sucrose (McLean and Cook, 1952). The material was then postfixed in a 1% osmium tetroxide (OsO_4) solution for $1\frac{1}{2}$ hours, followed by a rinse in cold water. The sample was then carried through a dehydration series of ethanol as follows : 25% for 5 minutes, 50% for 5 minutes, 75% for 5 minutes, 90% until room temperature was reached, and 100% with three changes, 10 minutes each.

Embedding was done in Spurr's low viscosity epoxy resin medium in polyethylene Beem capsules[2] (Spurr, 1969). After hardening overnight at 70 C, thin sections were cut with an LKB ultramicrotome[2] with thermal advance. Thickness of the sections was determined by their interference colors, when viewed under a dissecting microscope, according to the technique described by Peachy (1958). Silver to silver-gray thin sections (60 - 90 nm thick) were then mounted on unsupported 300 mesh copper grids. Staining was done with 0.5% uranyl acetate in 90% ethanol and Reynolds'

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lead hydroxide chelated with citrate (Reynolds, 1963 ; Dawes, 1971).

The thin sections were examined with an RCA EMU-3G electron microscope[3] at an electron acceleration of 50 kV, and magnifications ranging from 5,800 to 16,000 X. Micrographs of 5 cm x 5 cm were taken on Kodak Electron Microscopy Film[3] (Estar thick base) with extremely fine grain.

Gonadal Sperm Concentration

The concentration of spermatozoa in the three consecutive areas (anterior, medial, and posterior) of the spermatogenic part of the testes was determined. The spermatogenic part was blotted dry and divided into three equal lengths. The following measurements were taken on each section : weight (to the nearest 0.01 g), length (mm), and volume (to the nearest 0.01 cc). Determination of the volume was carried out by displacement in a 0.65% NaCl solution as previously described.

The three samples were handled separately following exactly the same procedures.

The testicular tissue was blotted dry, placed in a 2 cc vial and cut in very small pieces, then it was thoroughly teased to release the spermatozoa from the tissue. One cc of a 0.65% NaCl solution was added to the vial and the

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sample homogenized with a Virtis S23 microblender-homogenizer[4] for approximately 20 minutes at high speed.

To count the number of spermatozoa per cubic centimeter in this suspension, the standard technique for counting blood cells was used. While mixing the homogenate, two red blood cell pipettes were filled to the 0.5 mark and then diluted with a 0.65% NaCl solution to the 101 mark. This technique resulted in a 1 to 200 dilution of the homogenate.

The pipettes were then shaken automatically for approximately 5 minutes. A drop of the mixed suspension was released from the pipette on each of the two chambers of a hemacytometer. A Spencer Bright-Line improved Neubauer hemacytometer[4] with chambers of 0.1 mm depth was used. The sample was covered with a number 1 cover slip and examined under a phase contrast microscope at a magnification of 1,569 X. The total number of spermatozoa in 80 small squares of the chamber-grid was counted.

Two blood pipettes were prepared for each tissue sample and two chambers of the hemacytometer filled for each pipet. This procedure resulted in four replicated counts for each tissue sample. The mean of the four counts was calculated, and from this figure the total number of spermatozoa in the tissue suspension derived. The hemacytometer

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is built in such a manner that when the dilution in the blood cell pipettes is 1 to 200, and the number of cells in 80 small grid squares is counted, the number of cells per cubic millimeter in the tissue homogenate is 10^5 x the number of cells counted. From this number, the total number in the tissue suspension was calculated, and since the volume and weight of the testicular tissue that provided these spermatozoa was recorded, the number of spermatozoa per gram of wet testicular material could be determined.

Some variation existed in the original weights of the tissue samples among the three areas, therefore the weighed means of the three concentrations were used to calculate the overall gonadal sperm concentration. The spermatozoal concentration was expressed as spermatozoa x 10^9/g of wet testicular tissue.

Salinity and Temperature Influence on Spermatozoal Motility in Vitro

A separate experiment was conducted in May 1972, to determine the influence of salinity and temperature at regular time intervals on the motility of spermatozoa teased from the testes.

Four male channel catfish were used, one 2-year-old, and one 3-year-old of the wild stock, and one 2-year-old, and one 3-year-old of the domestic stock.

Spermatozoal motility was tested in salinities of 0, 2, 5, and 8°/$_{oo}$, each at respectively 24, 27, and 30 C. The saline solutions were made by adding commercial synthetic seasalts (Rila Marine Mix)[5] (Table 2) to fresh pond water. The salinities were determined by titration with mercuric nitrate, $Hg(NO_3)_2$ (Anonymous, 1965). The osmotic pressure of each saline solution was determined by freezing point depression with an Osmette[5] precision osmometer, and the pH with a Beckman[5] pH meter.

For each salinity a test tube containing 2 cc saline water was placed in each of three electric water baths, and maintained at respectively 24, 27, and 30 C. The test tubes containing the saline solutions were introduced into the water bath several hours prior to the beginning of each test. To allow the solution to reach the appropriate temperature and stabilize.

For each fish the same procedures were followed. The fish was stunned by electric shock, its body measurements taken and then it was dissected. A blood sample was collected from each fish by heart puncture, then centrifuged at 20 RPM for 30 minutes and the blood plasma's osmotic pressure determined with the Osmette[5].

Approximately 0.05 g of spermatogenic testicular tissue was placed in each test tube, and the tissue teased

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Table 2. Composition (ppm) of Rila Marine Mix[6] synthetic sea salts solution of approximate specific gravity 1.022 to 1.025 at 22.2 C.

NaCl	26,400.000
$MgSO_4$	6,050.000
$MgCl_2.6H_2O$	5,080.000
Ca (as Cl^-)	1,060.000
KCl	640.000
Ca (as $SO_4^=$)	300.000
KNO_3	100.000
$Na_2SiO_3.9H_2O$	100.000
KBr	22.000
KI	20.000
K_2HPO_4	10.000
Sr (as Cl^-)	3.800
Mn (as $SO_4^=$)	0.700
Zn (as $SO_4^=$)	0.200
Fe (as $SO_4^=$)	0.100
Co (as $SO_4^=$)	0.070
Tris (hydroxymethyl) aminomethane	0.040
Al (as Cl^-)	0.030
Mo (as NaMoO)	0.020
Pb (as $SO_4^=$)	0.010
Rb (as Cl^-)	0.006
Li (as Cl^-)	0.006
Cu (as $SO_4^=$)	0.001

apart. Introduction of the material in the saline solution proceeded in a sequential order, to allow assessment of the

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spermatozoal motility at the predetermined time intervals. The motility was estimated using the previously described method, and according to the criteria in Table 1, an evaluation was carried out at 1, 5, 20, 40, 60, 80, 100, 120, 140, 160, 180, 240, 300, 360, and 720 minutes after introduction of the tissue in the test tube.

A drop of the suspension was obtained from the appropriate test tube and placed on a glass slide. The slides were acclimated to the same temperature as the sample under examination, to avoid a temperature shock in the spermatozoa, which could influence their motility and bias the assessment (Mann, 1954). Room temperature during the tests was maintained at 24 C ; therefore, slides used in evaluating motility at 27 and 30 C were previously warmed to the appropriate temperature on an electric slide warmer.

Assessment and recording of the spermatozoal motility was completed within $\frac{1}{2}$ minute, after putting the suspension on the slide. This quick procedure limited the effects of room temperature and heating by the microscope light, on the motility.

Statistical Procedures

Some of the examined fish lacked testes, while others had well developed gonads, but without sperm. The partial data collected from these fish were excluded from the statistical procedures and handled separately. For some

analyses, the data obtained during November 1971 were discarded, because some observations for the 2-year-old domestic stock were missing. In these fish testes could not be recognized, although the secondary male sexual characteristics were present.

The data were analysed by least square analyses of variance, and corrected for unequal numbers within classes, which occurred in a few cases. The means of the different variables were calculated within and among each source of variation. The standard error of the mean, the standard deviation from the mean and the coefficient of variation were calculated and the range recorded.

The correlation coefficients between every two variables were computed, with the exception of those relating to the spermatozoal dimensions.

The date collected during the test on salinity and temperature influence on spermatozoal motility in vitro, were also analyzed by least square analysis of variance. The mean motility score was computed within and among each of the four sources of variation (fish, temperature, salinity, time).

RESULTS AND DISCUSSION

Fish Lacking Testes and Fish with Testes Devoid of Spermatozoa

A total of 48 male channel catfish were examined during the 6 months study (November 1971 - April 1972). Of these fish, six had not yet developed testes, although some had a well-developed male genital papilla and pronounced male secondary sexual characteristics. Öztan (1954) observed a similar situation in Anatolian cyprinodonts. Male hybrids between the genera Kosswigichthys, Aphanius, and Anatolichthys, were sterile, some having reduced testes, but all expressed their secondary sexual characteristics normally with respect to color and behavior. The channel catfish lacking testes were small, 2-year-olds, belonging to both the Lake des Allemands and Yazoo City stocks.

In Table 3 the month each fish was examined, its stock, body measurements and a comment regarding the protrusility of its genital papilla, are given.

Reaching sexual maturity is dependent on the age and the size of fishes, with some variation among individuals of a species (Lagler et al., 1962). Although the six channel catfish were old enough to be sexually mature, four

Table 3. Body measurements and comments regarding the protrusility of the genital papilla of six 2-year-old male channel catfish devoid of testes, identified by month of examination and by stock

Month	Stock	Body		Head		Protrusility general papilla
		Weight (g)	Total length (mm)	Width (mm)	Circumference (mm)	
Nov. '71	D.A.[a]	256	301	47	135	Pronounced
Nov. '71	Yazoo[b]	125	271	38	108	Pronounced
Nov. '71	Yazoo	105	254	34	108	Unpronounced
Dec. '71	D.A.	58	206	28	89	Unpronounced
Jan. '72	D.A.	130	266	38	110	Pronounced
March '72	Yazoo	381	352	48	132	Pronounced
	Mean	176	275	39	113	

[a] Wild fish from Lake des Allemands, Louisiana.

[b] Domestic fish from Yazoo City, Mississippi.

were undersized, which probably was the overriding factor for lacking testes. Fishes in the size category of the two larger ones (256 and 381 g) usually have developed testes at $1\frac{1}{2}$ or 2 years but individual variation exists.

Besides these six fish devoid of testes, two cases were encountered where 3-year-old channel catfish had very well developed male secondary sexual characteristics and testes, but no spermatozoa were produced.

In Table 4 the body and testes measurements and their means of these two fish are presented.

Normally the anterior spermatogenic part of channel catfish testes is white, lobular and consists of very soft tissue, while the posterior, glandular part is pinkish, with very fine lobules and harder tissue (Sneed and Clemens, 1963). In the domestic fish, exhibiting azoospermia, the anterior part of the testes was pinkish, with very fine lobules and hard tissue. It had the morphological appearance of the glandular part, and was difficult to distinguish.

The testes of the wild azoospermic fish, however, appeared normal in all respects.

For both fish, several tissue samples were taken, throughout the testes, and examined under the light microscope, but no spermatozoa could be detected.

The process of spermatogenesis is under endocrine control, and imbalance in hormonal composition may be responsible for inhibiting spermatogenesis, resulting in

Table 4. Body and testes measurements and gonosomatic index of two 3-year-old sterile male channel catfish, identified by month of examination and by stock

	Dec. '71 Yazoo[a]	Feb. '72 D.A.[b]	Mean
Body			
Weight (g)	1240	517	884
Length (mm)	512	375	448
Head			
Width (mm)	80	57	68
Circumference (mm)	230	155	192
Testes			
Weight (g)	2.40	1.17	1.78
Length (mm)	15	89	52
Volume (cc)	2.30	1.14	1.72
Gonosomatic index	0.19	0.23	0.21
Part of testes normally spermatogenic, here azoospermic			
Weight (g)	1.24	0.84	1.04
Length (mm)	100	65	82
Volume (cc)	1.20	0.80	1.00
Width (mm)	16	20	18
Glandular part of testes			
Weight (g)	1.16	0.33	0.74
Length (mm)	50	24	37
Volume (cc)	1.10	0.34	72
Width (mm)	10	10	10

[a] Fish from Yazoo City, Mississippi.

[b] Fish from Lake des Allemands, Louisiana.

infertility. Sundararaj et al. (1967) restored spermatogenesis in previously hypophysectomized catfish, Heteropneustes fossilis (Bloch) by injecting them with follicle-stimulating hormone (FSH) and human chorionic gonadotrophin (HCG), separately or in combination.

The causes of spermatogenesis inhibition are complex and differ from one species to the other. To ascertain the reason for azoospermia in the channel catfish, an extensive study of its endocrine processes would be necessary.

Body and Testes Measurements, Gonosomatic Index, and Gonadal Sperm Concentration

Secondary sexual characteristics were usually more pronounced in the larger fish, which were also the older ones. No apparent differences among stocks could be identified. In all fish examined, the male genital papilla and a relatively wide muscular head could be recognized, although in some individuals these characteristics were more pronounced than in others. The dark pigmentation of the lower mandibular region however, was only evident in the larger fish.

In Table 5 the overall mean, standard error of the mean, coefficient of variation, and range of the body and testes measurements are given, as well as the gonosomatic index and gonadal sperm concentration, pertaining to all the normal channel catfish examined in the study.

Table 5. Overall mean, standard error of the mean, coefficient of variation and range of body and testes measurements, gonosomatic index, and gonadal sperm concentration of 40 male channel catfish, all months (November 1971-April 1972), stocks, and ages combined

Variable	Number of observations	Mean	Standard error	Coefficient of variation	Range
Body					
Weight (g)	40	687	50	45.84	142-2050
Total length (mm)	40	405	7	10.68	265-582
Head					
Width (mm)	40	66	2	17.12	35-120
Circumference (mm)	40	182	7	24.21	65-290
Testes					
Weight (g)	40	1.69	0.16	59.33	0.09-6.87
Length (mm)	40	102	3	22.06	30-187
Volume (cc)	40	1.70	0.16	57.89	0.08-6.70
Gonosomatic index (%)	40	0.22	0.01	39.55	0.05-0.62
Gonadal sperm concentration (10^9 spermatozoa/g wet testicular tissue)	40	5.4957	0.3279	37.73	1.6896-9.745

More detailed information is shown in Table 6, where the mean of the variables and their standard error are grouped by month the examination took place, by fish stock, and by age.

In Appendix table 1 the analyses of variance for each of the variables is summarized, giving all sources of variation, the degrees of freedom, the mean squares and levels of statistical significance.

Differences among months were significant ($P < 0.05$) for the means of total testes weight and volume only. From November 1971 through February 1972 the means fluctuated little, but a substantial increase was apparent for March and April, almost doubling some of the values obtained during the fall and winter. The spawning season of channel catfish begins when water temperature reaches approximately 21 C (Anonymous, 1970b), which in the southern United States occurs in early May. The increase in gonadal material in early spring probably continues until spawning activities commence. Gonadal maturation is enhanced by the combination of longer day lengths and warmer water temperatures. The gonadal cycle is also reflected in the increase of the gonosomatic index during early spring. Although a highly significant difference ($P < 0.01$) among months was obtained for the gonosomatic index of the males, the values varied only between 0.05 and 0.62% among individuals, and fluctuated between 0.16 and 0.32% when

Table 6. Mean and standard error of the mean of body and testes measurements, gonosomatic index and gonadal sperm concentration of 40 male channel catfish, grouped by month, by stock, and by age

Number of observ.	Month	Stock	Age	Body		Head		Testes			Gonosom. index (%)	Gonadal sperm concentration[a]
				Weight (g)	Total length (mm)	Width (mm)	Circ. (mm)	Weight (g)	Length (mm)	Volume (cc)		
5	Nov.'71	Both	Both	681±141	419±19	64±5	172±20	1.23±0.45	108±10	1.53±0.44	0.18±0.04	6.1777±0.9273
6	Dec.'71	Both	Both	587±129	327±18	61±5	170±18	1.40±0.41	86± 9	1.36±0.40	0.20±0.04	4.4339±0.8465
7	Jan.'72	Both	Both	671±119	406±16	65±4	175±17	1.22±0.38	91± 8	1.18±0.37	0.16±0.03	5.3844±0.7837
7	Feb.'72	Both	Both	596±119	394±16	59±4	183±17	1.29±0.38	96± 8	1.30±0.37	0.17±0.03	4.1697±0.7837
7	March'72	Both	Both	815±119	417±16	70±4	199±17	2.28±0.38	110± 8	2.24±0.37	0.27±0.03	5.1756±0.7837
8	April'72	Both	Both	749±111	419±15	73±4	189±16	2.45±0.36	118± 8	2.42±0.35	0.32±0.03	7.4036±0.7332
20	All	D.A.[b]	Both	474± 70	352±10	57±2	162±10	1.12±0.22	84± 5	1.10±0.22	0.21±0.02	5.3599±0.4637
20	All	Yazoo[c]	Both	901± 70	459±10	75±2	203±10	2.27±0.22	119± 5	2.31±0.22	0.24±0.02	5.6315±0.4637
18	All	Both	2[d]	377± 74	338±10	51±3	145±10	0.73±0.24	70± 5	0.72±0.23	0.17±0.02	3.9562±0.4888
22	All	Both	3[e]	941± 67	460± 9	78±2	213± 9	2.48±0.21	128± 5	2.51±0.21	0.27±0.02	6.7554±0.4421

[a] x 10^9 spermatozoa/g wet testicular tissue.

[b] Wild fish from Lake des Allemands, Louisiana.

[c] Domestic fish from Yazoo City, Mississippi.

[d] Fish in their second year.

[e] Fish in their third year.

however, the gonosomatic index increased. Jerald and Brown (1969) calculated the gonosomatic index for channel catfish from Lake Carl Blackwell in Oklahoma. Minor fluctuations of gonosomatic index of the males were observed during the fall and winter, but beginning in April through July a steady increase occurred, reaching a maximum of approximately 0.80% in July and decreasing rapidly thereafter.

For channel catfish, the seasonal gonadal cycle is less pronounced than for some other fish species. In a recent study, Kaya and Hasler (1972) calculated the gonosomatic index of male green sunfish, Lepomis cyanellus Rafinesque. The index remained below 0.50% from mid-fall to early spring but rapidly increased during late spring. A maximum of approximately 4.50% was reached at the end of June, followed by a rapid decrease to 0.50% by August.

Billard et al. (1971) calculated the gonosomatic index for male rainbow trout, Salmo gairdneri Richardson. At the onset of the spawning, the average index value was 3%, but declined to approximately 0.75% 3 months afterwards.

Highly significant differences ($P < 0.01$) were obtained between the mean body and testes measurements when either stocks or ages were compared.

The domestic fish were much larger than the wild ones. Originally, equal numbers of fry were stocked per unit area, in separate adjacent ponds, for both stocks, but fish from

Lake des Allemands had much better survival compared to the Yazoo City fish.

When the fish are grouped by age regardless which stock they belong to, the 3-year-olds outperform the 2-year-olds, in mean body and testes dimensions as well as in gonosomatic index and gonadal sperm concentration.

Percent Live Spermatozoa and Motility Score

An average of 73.2% of the spermatozoa teased from the testes were alive (Table 7). When comparing the three consecutive areas of the spermatogenic part of the testes, a slight increase in percent living gametes occurred from the anterior to the posterior area (Tables 8 and 9). This was the only significant ($P < 0.05$) source of variation (Appendix table 2). Mean values of percent live spermatozoa seemed independent of month, stock and age effects (Table 9 and Appendix table 2).

Spermatozoal motility was generally high, with most cells progressively motile and some vibrating in loco (Table 7). When comparing the mean motility by month, December ranked highest, with a 4.00 score (Table 9 and Appendix table 2). Motility values were similar among the three areas of the spermatogenic part of the testes (Tables 8 and 9), as well as among stocks and ages (Table 9).

Table 7. Overall mean, standard error of the mean, and coefficient of variation of percent live spermatozoa and motility score of 35 male channel catfish, all months (December 1971-April 1972), stocks, ages, and testes areas combined

Variable	Number of observations	Mean	Standard error	Coefficient of variation
Live spermatozoa (%)	105	73.2	0.60	8.33
Motility score	105	3.27	0.04	11.64

Table 8. Mean, standard deviation, and range of percent live spermatozoa and motility score in the three testes areas of 40 male channel catfish, all months (November 1971-April 1972), stocks, and ages combined

Variable	Number of observations	Mean	Standard deviation	Range
Live spermatozoa (%)				
Anterior area	40	67.4	17.2	3.5 - 97.5
Medial area	40	68.7	17.6	0.0 - 89.0
Posterior area	40	72.3	18.0	4.5 - 94.0
Motility score				
Anterior area	40	3.32	0.94	1.00 - 4.00
Medial area	40	3.32	0.92	1.00 - 4.00
Posterior area	40	3.35	0.89	1.00 - 4.00

Table 9. Mean and standard error of the mean of percent live spermatozoa and motility score of 35 male channel catfish, grouped by month, by stock, by age, and by testes area

Number of observations	Month	Stock	Age	Testes area	Live spermatozoa (%)	Motility score
18	Dec. '71	Both	Both	All	68.5 ± 5.2	4.00 ± 0.22
21	Jan. '72	Both	Both	All	79.7 ± 4.8	3.05 ± 0.20
21	Feb. '72	Both	Both	All	73.4 ± 4.8	3.48 ± 0.20
21	March '72	Both	Both	All	69.1 ± 4.8	2.33 ± 0.20
24	April '72	Both	Both	All	74.5 ± 4.5	3.54 ± 0.19
51	All	D.A.[a]	Both	All	74.0 ± 3.1	3.10 ± 0.13
54	All	Yazoo[b]	Both	All	72.5 ± 3.0	3.42 ± 0.12
51	All	Both	2[c]	All	74.9 ± 3.1	3.37 ± 0.13
54	All	Both	3[d]	All	71.6 ± 3.0	3.17 ± 0.12
35	All	Both	Both	Anterior	71.3 ± 1.0	3.26 ± 0.06
35	All	Both	Both	Medial	72.8 ± 1.0	3.26 ± 0.06
35	All	Both	Both	Posterior	75.6 ± 1.0	3.28 ± 0.06

[a] Wild fish from Lake des Allemands, Louisiana.

[b] Domestic fish from Yazoo City, Mississippi.

[c] Fish in their second year.

[d] Fish in their third year.

Testes Measurements

The testes of catfishes (Ictaluridae) and accessory reproductive glands have been described by Sneed and Clemens (1963).

Measurements of the testes in toto were previously presented in Tables 5 and 6. Since two distinct parts, a spermatogenic and a glandular one exists, attention was given to both individually. Table 10 shows the statistics of the measurements when spermatogenic and glandular parts were combined, while in Table 11, the mean values of all measurements are represented for each part separately.

Weight- and volume-wise, the spermatogenic part made up approximately 1/2 of the total testes, but extended over 2/3 of the total length of the male gonads (Table 11). Sneed and Clemens (1963), reported the anterior 3/4 of the testes of the Ictaluridae as being spermatogenic, while the posterior 1/4 did not produce or contain spermatozoa. Histological sections revealed that the spermatogenic part consisted of cuboidal cells in various stages of division and also contained mature spermatozoa, while the lobes of the glandular part were lined with columnar epithelium. These authors suggest a secretive function of the glandular part, which is not essential to the viability of the spermatozoa.

The two parts can be easily recognized in most cases, by their color, morphology and tissue texture. The sper-

Tabel 10. Overall mean, standard error of the mean, and coefficient of variation of measurements of the spermatogenic and glandular parts of the testes of 35 male channel catfish, all months (December 1971-April 1972), stocks, ages, and testes parts combined

Variable	Number of observations	Mean	Standard error	Coefficient of variation
Testes part				
Weight (g)	70	0.88	0.05	48.41
Length (mm)	70	50	1	19.34
Volume (cc)	70	0.86	0.05	49.00
Width (mm)	70	16	0.5	26.20

Table 11. Mean, standard deviation, and range of measurements of spermatogenic and glandular parts of the testes of 40 male channel catfish, all months (November 1971-April 1972), stocks and ages combined

Variable	Number of observations	Mean	Standard deviation	Range
Spermatogenic part				
Weight (g)	40	1.14	0.95	0.04 - 3.68
Length (mm)	40	68	26	18 - 120
Volume (cc)	40	1.13	0.94	0.04 - 3.70
Width (mm)	40	18	9	4 - 36
Glandular part				
Weight (g)	40	0.56	0.68	0.03 - 3.74
Length (mm)	40	34	20	12 - 87
Volume (cc)	40	0.57	0.69	0.04 - 3.60
Width (mm)	40	13	7	2 - 30

matogenic part is white, with rather broad lobules and consists of mushy tissue, while the glandular part is pink, with very fine lobules of less soft tissue. Sneed and Clemens (1963) stated that the spermatogenic part became whiter as the spawning season approached. In our study, no seasonal changes in pigmentation were observed, but some color variation existed among individuals. Some fish had very white spermatogenic lobules, while in other a pinkish tone appeared. The glandular part, however, was pink in all the examined fish, with little tone variation among specimens.

Sneed and Clemens (1963) also reported that the spermatogenic part progressively enlarges at the approachment of the spawning season. These observations were made from testes collected during January, April, and July. In our study, an increase in both parts was noticed, starting in March and continuing through April (Tables 12 and Appendix table 3). During the late fall and winter, however, the means of the testes measurements fluctuated from month to month around the same values, without any apparent trend.

The fish from Yazoo City, Mississippi had considerably larger gonads than the fish from Lake des Allemands, when absolute values were compared. The domestic fish were also larger in size (Table 6), which probably accounted for most of the larger values. The gonosomatic index, which gives the weight of the testes relative to the body weight, was not significant ($P > 0.05$) among stocks (Appendix table 1).

Table 19. Mean and standard error of the mean of measurements of spermatogenic and glandular parts of the testes of 35 male channel catfish, grouped by months, by stock, and by age

Number of observ.	Month	Stock	Age	Spermatogenic part				Glandular part			
				Weight (g)	Length (mm)	Volume (cc)	Width (mm)	Weight (g)	Length (mm)	Volume (cc)	Width (mm)
6	Dec.'71	Both	Both	1.00±0.17	52±4	0.98±0.17	15±2	0.40±0.17	34±4	0.38±0.17	11±2
7	Jan.'72	Both	Both	0.91±0.16	63±4	0.89±0.16	17±2	0.31±0.16	28±4	0.30±0.16	12±2
7	Feb.'72	Both	Both	0.82±0.16	62±4	0.82±0.16	16±2	0.47±0.16	32±4	0.46±0.16	12±2
7	March'72	Both	Both	1.52±0.16	77±4	1.51±0.16	20±2	0.76±0.16	33±4	0.74±0.16	14±2
8	April'72	Both	Both	1.48±0.15	78±3	1.48±0.15	22±1	0.96±0.15	40±3	0.94±0.15	18±1
17	All	D.A.[a]	Both	0.87±0.10	60±2	0.86±0.10	16±1	0.31±0.10	26±2	0.30±0.10	12±1
18	All	Yazoo[b]	Both	1.44±0.10	74±2	1.42±0.10	20±1	0.87±0.10	41±2	0.84±0.10	14±1
17	All	Both	2[c]	0.50±0.10	48±2	0.49±0.10	12±1	0.24±0.10	21±2	0.22±0.10	9±1
18	All	Both	3[d]	1.79±0.10	86±2	1.77±0.10	25±1	0.94±0.10	45±2	0.92±0.10	18±1

[a] Wild fish from Lake des Allemands, Louisiana.

[b] Domestic fish from Yazoo City, Mississippi.

[c] Fish in their second year.

[d] Fish in their third year.

The individual correlation coefficients, however, between the fishes body measurements (body weight, and total length of the body) and the measurements of the total testes, or the individual testicular parts (weight, length and volume), were all highly significant ($P < 0.01$), ranging from 0.73 to 0.91 (Appendix table 7).

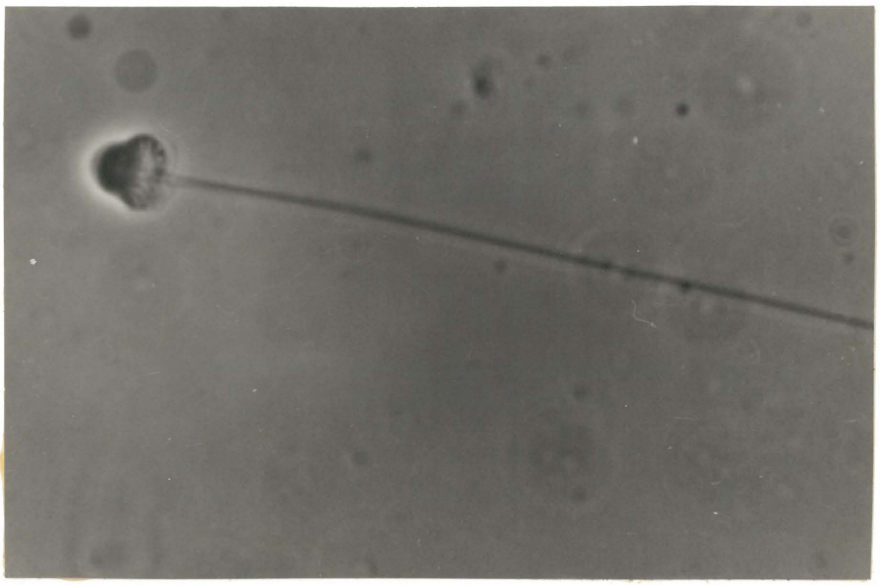
Three-year-old fish as a group had also considerably higher mean absolute values for the testes dimensions, compared to the 2-year-old ones (Table 12 and Appendix table 3). Some of the difference could probably be attributed to a size effect. Since the gonosomatic index showed a highly significant difference ($P < 0.01$) between the two age groups (Appendix table 1), the body size effect should, however, not be overrated.

Spermatozoal Morphology

The spermatozoon of channel catfish comprises a rounded head, a collar-like midpiece and a long flagellum. Figure 1 shows the gross morphology of a spermatozoon, approximately 5,020 X enlarged. The head appears dark, due to the dense nuclear chromatin, while the collar-like midpiece, containing the mitochondria and cytoplasma is lighter in color. Approximately 1/3 of the total flagellum is shown in Figure 1.

Mattei (1969) has given a detailed account on comparative spermatogenesis and spermatozoal morphology among 82 fish species, but not including Ictaluridae. In

Figure 1. Photograph of a normal spermatozoon of channel catfish, magnified 5,020 X (only 1/3 of the flagellum is shown).



gross morphology, the channel catfish spermatozoon resembles the schematic drawing of the male gamete of Clarias senegalis, belonging to the same order of Siluriformes as the Ictaluridae (Mattei, 1969).

Great morphological and structural diversity exists in fish spermatozoa. Teleost species examined until now, only share one characteristic, namely the absence of a true acrosome (Mattei, 1969 ; Nicander, 1969).

Stains

Neither the rose Bengal nor the fast green stain gave satisfactory results with channel catfish spermatozoa. With rose Bengal the staining was very patchy and most cells were destroyed. In the fast green stain the spermatozoa seemed to remain intact but only an overall green hue of the preparation was obtained. The pinacyanol chloride stain seemed to slightly sharpen the spermatozoal contour. Although this stain gave good results with spermatozoa of several fish species (Cruea et al., 1969), its use offered no real advantages in this study.

Staining procedures can alter the chemical composition of cells, they can result in the formation of artifacts and are time consuming (De Robertis et al., 1965). Since unstained spermatozoa could be easily observed under the phase contrast microscope, it was preferred to examine living cells only.

Dimensions

In Table 13 the mean dimensions of channel catfish spermatozoa are presented. The values obtained for length and width of the head, are of the same magnitudes than those reported by Billard (1969) for four teleost fishes. Billard (1969) measured the following spermatozoon dimensions, for respectively the length of the head and its maximal width : 3.3 µm and 2.5 µm for common carp, Cyprinus carpio Linnaeus ; 2 µm and 1.8 µm for the Northern pike, Esox lucius Linnaeus ; 2.5 µm and 1.5 to 2 µm for rainbow trout and brown trout, Salmo trutta fario Linnaeus; 4 µm and 1 µm for guppy, Poecilia reticulata Peters.

Little variation in the mean dimensions occurred as is shown in Table 14 for observations grouped by month, by stock, by age, and by area of the testes. Although in the analyses of variance summary (Appendix table 4) a significant difference ($P < 0.05$) among months is shown for some of the spermatozoon dimensions, these were in reality so small, relating to a fraction of a micrometer, that for all practical purposes, they can be disregarded because of human error and limited accuracy of the ocular micrometer.

Aberrations

For all the fish examined, an average of 14.1% of the spermatozoa showed morphological aberrations of the flagellum (Table 15). In evaluating the percentage of abnormal spermatozoa, two morphological tail aberrations were

Table 13. Overall mean, standard error of the mean and coefficient of variation of spermatozoan dimensions of 35 male channel catfish, all months (December 1971-April 1972), stocks, ages, and testes areas combined

Variable	Number of observations	Mean	Standard error	Coefficient of variation
		μm		
Total length head & midpiece	525	3.9	0.01	7.64
Length head	525	2.3	0.01	10.29
Length midpiece	525	1.6	0.01	12.94
Width head	525	2.4	0.01	8.21
Width midpiece	525	3.1	0.01	8.09
Length flagellum	525	94.9	0.26	6.38

Table 14. Mean and standard error of the mean of spermatozoan dimensions of 35 male channel catfish, grouped by month, by stock, by age and by testes area

Number of observ.	Month	Stock	Age	Testes area	Total length head & midpiece	Length head	Length midpiece	Width head	Width midpiece	Length flagellum
					μm					
90	Dec.'71	Both	Both	All	3.9±0.02	2.4±0.02	1.5±0.02	2.4±0.01	3.2±0.02	96.3±0.58
105	Jan.'72	Both	Both	All	3.9±0.02	2.3±0.02	1.6±0.02	2.3±0.01	3.1±0.02	93.4±0.54
105	Feb.'72	Both	Both	All	3.9±0.02	2.3±0.02	1.6±0.02	2.4±0.01	3.1±0.02	94.8±0.54
105	March'72	Both	Both	All	3.9±0.02	2.3±0.02	1.6±0.02	2.4±0.01	3.1±0.02	95.8±0.54
120	April'72	Both	Both	All	3.9±0.02	2.4±0.02	1.5±0.02	2.4±0.01	3.1±0.02	94.6±0.50
255	All	D.A.[a]			3.9±0.01	2.4±0.01	1.6±0.01	2.4±0.01	3.1±0.01	95.6±0.35
270	All	Yazoo[b]			3.9±0.01	2.3±0.01	1.6±0.01	2.4±0.01	3.1±0.01	94.4±0.34
255	All	Both	2[c]	All	3.9±0.01	2.4±0.01	1.6±0.01	2.4±0.01	3.1±0.01	95.3±0.35
270	All	Both	3[d]	All	3.9±0.01	2.3±0.01	1.6±0.01	2.4±0.01	3.1±0.01	94.6±0.34
175	All	Both	Both	Ant.	3.9±0.02	2.3±0.02	1.6±0.02	2.4±0.02	3.2±0.02	94.8±0.46
175	All	Both	Both	Med.	3.9±0.02	2.3±0.02	1.6±0.02	2.4±0.02	3.1±0.02	95.0±0.46
175	All	Both	Both	Post.	3.9±0.02	2.3±0.02	1.5±0.02	2.4±0.02	3.1±0.02	94.9±0.46

[a] Wild fish from Lake des Allemands, Louisiana.

[b] Domestic fish from Yazoo City, Mississippi.

[c] Fish in their second year.

[d] Fish in their third year.

Table 15. Overall mean, standard error of the mean and coefficient of variation of percent normal spermatozoa and percent spermatozoa with morphological aberrations of 35 male channel catfish, all months (December 1971-April 1972), stocks, ages, and testes areas combined.

Variable	Number of observations	Mean	Standard error	Coefficient of variation
Normal spermatozoa (%)	105	85.9	0.6	6.77
Biflagellar spermatozoa (%)	105	4.7	0.2	37.98
Spermatozoa with split flagellum (%)	105	9.4	0.4	46.06

recognized : the occurrence of two distinct flagella and an apparently longitudinally split flagellum, of which both parts join in some areas of the tail and separate in others. The existence of two flagella arising separately from the head region, was confirmed by examination under the electron microscope. The split flagellum, however, could actually consist of two separate tails, overlapping each other at some points.

Salisbury and VanDemark (1961) and Hafez (1968) distinguish between primary and secondary bovine sperm cell aberrations. Primary abnormalities are a result of faulty spermatogenic processes, whereas secondary malformations take place during passage of the spermatozoa in the male ducts, or is caused by handling sperm in vitro. The aberrations observed in channel catfish were probably for the most part primary abnormalities, although some mechanical injury could have occurred during examination.

Occasionally, spermatozoa with wide round heads were encountered, but this was probably the result of water absorption and swelling and not a morphological abnormality.

Among the three consecutive areas in the spermatogenic part of the testes, little variation in the relative percentages of normal and abnormal spermatozoa existed (Tables 16, 17 and Appendix table 5). A highly significant difference ($P < 0.01$) in the mean percent normal spermatozoa was apparent during March and April, coupled with a decrease in the number of spermatozoa exhibiting a split

Table 16. Mean, standard deviation and range of percent normal spermatozo and percent spermatozoa with morphological aberrations, in the three testes areas, of 40 male channel catfish, all months (November 1971-April 1972), stocks, and ages combined

Variable	Number of observ.	Mean (%)	Standard deviation (%)	Range (%)
Normal spermatozoa				
Anterior area	40	84.3	12.1	43.0-97.0
Medial area	40	85.3	11.3	49.5-97.0
Posterior area	40	84.2	12.8	41.0-97.0
Biflagellar spermatozoa				
Anterior area	40	4.8	3.5	1.0-17.0
Medial area	40	4.6	4.0	0.0-23.0
Posterior area	40	5.1	3.9	1.5-22.0
Spermatozoa with split flagellum				
Anterior area	40	10.9	9.9	1.0-45.0
Medial area	40	10.2	9.2	1.0-42.5
Posterior area	40	10.7	10.7	1.0-47.0

Table 17. Mean and standard error of the mean of percent normal spermatozoa and percent spermatozoa with morphological aberrations of 35 male channel catfish grouped by month, by stock, by age and by testes area

Number of observ.	Month	Stock	Age	Testes area	Normal spermatozoa	Biflagellar spermatozoa	Spermatozoa with split flagellum
					(%)		
18	Dec.'71	Both	Both	All	70.0±2.5	8.1±1.6	21.9±1.7
21	Jan.'72	Both	Both	All	86.0±2.3	6.0±1.5	8.1±1.6
21	Feb.'72	Both	Both	All	84.1±2.3	4.3±1.5	11.7±1.6
21	March'72	Both	Both	All	91.4±2.3	3.4±1.5	5.2±1.6
24	April'72	Both[a]	Both	All	94.5±2.2	2.5±1.4	3.0±1.5
51	All	D.A.[b]	Both	All	84.4±1.5	5.4±0.9	10.2±1.0
54	All	Yazoo[b]	Both	All	87.3±1.4	4.0±0.9	8.7±1.0
51	All	Both	2[c]	All	84.6±1.5	4.7±0.9	10.7±1.0
54	All	Both	3[d]	All	87.1±1.4	4.6±0.9	8.2±1.0
35	All	Both	Both	Ant.	85.1±1.0	4.8±0.3	10.1±0.7
35	All	Both	Both	Med.	86.8±1.0	4.4±0.3	8.8±0.7
35	All	Both	Both	Post.	85.8±1.0	4.8±0.3	9.3±0.7

[a] Wild fish from Lake des Allemands, Louisiana.

[b] Domestic fish from Yazoo City, Mississippi.

[c] Fish in their second year.

[d] Fish in their third year.

flagellum (Table 17). The domestic stock showed a significantly higher percentage of normal gametes, compared to the wild fish (Table 17 and Appendix table 5).

In fishes little attention has been given to the occurrence of spermatozoal aberrations and their possible effect on fertilization capacity of the milt.

Billard and Fléchon (1969) stated that occasionally two flagella occurred on the spermatids of guppy. Biflagellar spermatids were also observed in plainfin midshipman, Porichthys notatus Girard (Stanley, 1965) and in an African bichir, Protopterus annectens (Owen), a lungfish (Boisson et al., 1968). The number of flagella seems to be less consistent in fishes, than in other animal groups. The only known vertebrate to date, to have a spermatozoon lacking a flagellum, is the African teleost Gymnarchus niloticus (Cuvier) (Mattei et al., 1967a).

Mattei (1969) concluded from extensive studies that the spermatozoa of teleost fishes, resemble more those of bivalve mollusks, annelids and echinoderms, than the spermatozoa of other fishes.

Ultrastructure

Studies of spermatozoal ultrastructure have been made of several fishes including among others : Squalus suckleyi (Girard) (Stanley, 1964); European lamprey, Lampetra planeri Bloch (Follenius, 1965; Stanley, 1967); guppy (Mattei and Boisson, 1966; Billard, 1969, 1970); and tidepool sculpin,

Oligocottus maculosus Girard (Stanley, 1969).

The ultrastructures of Ictaluridae have, however, never been investigated and a first attempt was made in this study (Figures 2, 3, 4, and 5).

Head

Figure 2 is an electron micrograph of a normal spermatozoon of channel catfish.

Most of the rounded head consisted of dense nuclear chromatin, which contains the DNA during interphase (De Robertis et al., 1965). In channel catfish the chromatin was slightly granular (Figures 2 and 3) like in common carp. In rainbow trout, brown trout and Northern pike chromatin occurs as large granules, while in guppy it appears as an almost homogenous mass (Billard, 1969). The nuclear membrane was only slightly undulated.

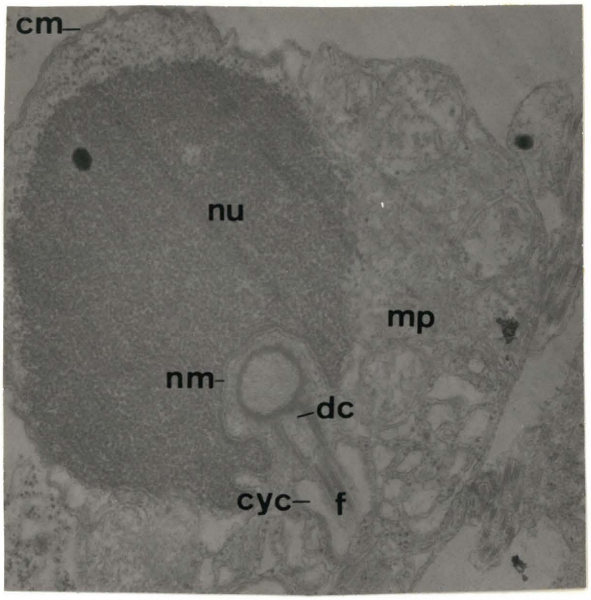
An acrosome was apparently lacking, which to date is the only characteristic common to spermatozoa of all teleost fishes (Porte and Follenius, 1960; Ginsburg, 1963; Stanley, 1969). The acrosome plays a crucial role in the fertilization process, which involves the penetration of the egg membrane by the spermatozoon, the attachment and fusion of the gametes, and activation of the egg (Franklin, 1970). Pasteels (1965a, 1965b) suggested that the absence of an acrosome might be associated with the presence of a micropyle in the eggs of teleost fishes.

List of abbreviations of Figures 2, 3, 4, and 5

cfi	central fibril
ch	chromatin
cm	cytoplasmic membrane
cyc	cytoplasmic canal
dc	distal centriole
f	flagellum
mi	mitochondrion
mp	midpiece
nm	nuclear membrane (karyotheca)
nu	nucleus
ofi	outer fibril
pc	proximal centriole
v	vesicle

Figure 2. Electron micrograph of a longitudinal section of the head region, of a normal spermatozoon of channel catfish, magnified 19,360 X.

Figure 3. Electron micrograph of a longitudinal section of the head region, of a biflagellar spermatozoon of channel catfish, magnified 19,690 X.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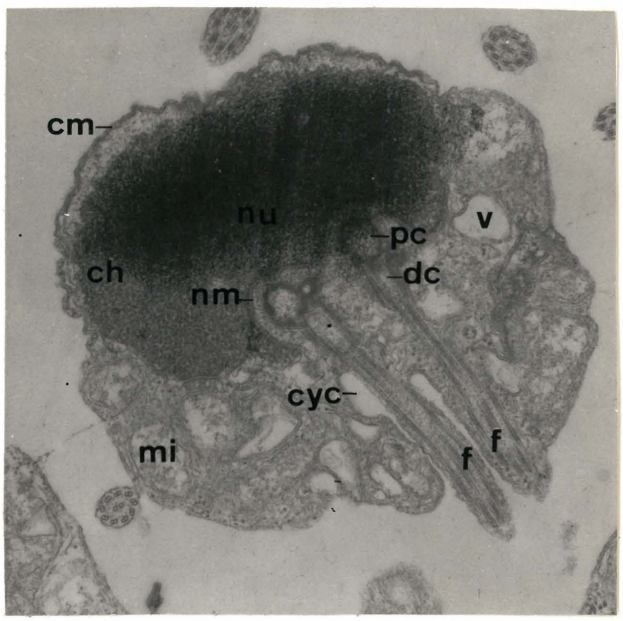
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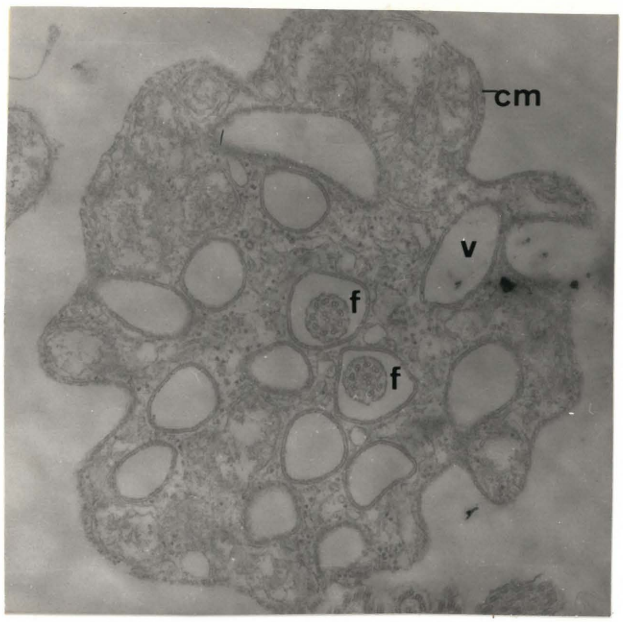
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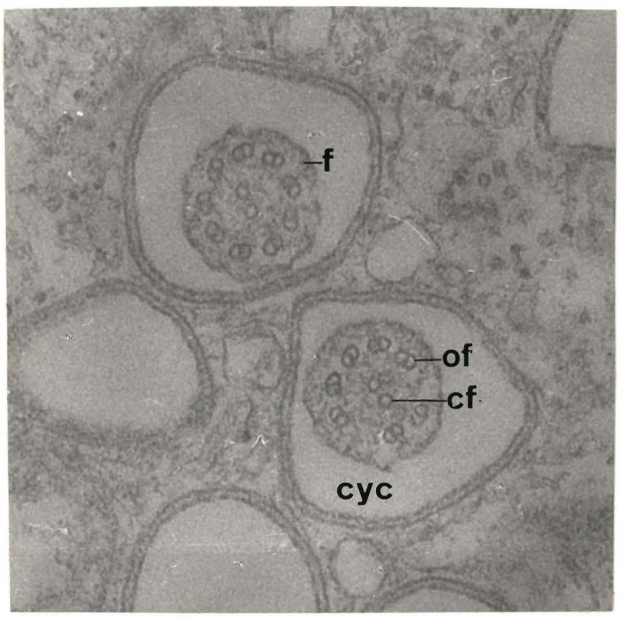
f
of
cf
cyc

Midpiece

A midpiece with collar-like appearance such as in channel catfish spermatozoa is usually referred to as cytoplasmic collar, or mitochondrial collar. In channel catfish the cytoplasmic collar was very broad, compared to the ones in carp, trouts, Northern pike and guppy (Billard, 1969).

The morphology of the cytoplasmic collar in teleosts might be associated with fertilization. Low collars occur if fertilization is external, and high ones in viviparous species (Porte and Follenius, 1960; Dadone and Narbaitz, 1967; Stanley, 1969).

The flagellum of the channel catfish spermatozoon was separated from the mitochondrial collar by an invagination of the cytoplasmic membrane, forming a cytoplasmic canal comparable to that of mammalian spermatids (Nicander, 1968; Billard, 1969, 1970) (Figures 2, 3, 4 and 5).

The midpiece contained mitochondria and vesicles, irregularly distributed in the cytoplasma.

Centriolar complex

The centriolar complex of spermatozoa in vertebrates consists usually of two structures, a proximal and a distal centriole, instead of the single basal body as in cilia. Structural modifications occur in either or both parts. In channel catfish spermatozoa, a distal centriole could be identified (Figures 2 and 3), and possibly a proximal

centriole (Figure 3). In common carp, the proximal centriole is present in the spermatid, but disappears during spermiogenesis (Billard, 1969). A more detailed study concentrating on the centriolar complex of channel catfish would be necessary to determine its structural properties.

Flagellum

The spermatozoal flagellum of channel catfish possesses the classical (2.9 + 2) axoneme of nine double outer fibrils (A and B) and two smaller, single central fibrils (Figures 3 and 4). The flagellum is bisymmetrical because of the nine double periferal fibrils. A plane perpendicular to the one through the two central fibrils, always cuts through one of the outer fibrils. This bisymmetry is probably associated with the two dimentional wave of the beating flagellum (Bishop, 1962). The flagellum lacked a periferal sheet or ridge which is usually formed by a fold in the tail plasma and oriented in a plane through the two central fibrils. The morphology of the ridges may vary along the length of the flagellum and be symmetrical or not. Spermatozoal ridges occur in the following fish species : silver salmon, Oncorhynchus kisutch (Walbaum) (Lowman, 1953); guppy (Mattei et al., 1967b); common carp, Northern pike, rainbow and brown trout (Billard, 1969); tidepool sculpin (Stanley, 1969).

Biflagellar spermatozoa were common for channel catfish, as previously reported. Each flagellum arose from an individual centriolar complex (Figure 3) and was separated from the cytoplasma of the midpiece and from one another, by a cytoplasmic canal (Figures 3, 4, and 5). In Figure 3, the cytoplasmic canal seemed to be absent between the flagellum on the right hand side, and the midpiece. Possibly this occurred due to compression of the tissue during sectionning, or it might be a structural aberration. It was observed only once during the study. In biflagellar spermatozoa, the central fibrils in the axoneme have identical orientation. The same fact has been reported by Stanley (1965) in biflagellar spermatozoa of plainfin midshipman. Similar orientation of the central fibrils occurs in cilia of the same cell and are associated with synchronisation of ciliary movements. The cilia beat in a plane perpendicular to the plane through the two fibrils (De Robertis et al., 1965). In biflagellar channel catfish spermatozoa this orientation could play a role in the mechanism of motility.

Spermatozoa of fishes are very diversified in their general and ultrastructural morphology. Billard (1969) distinguishes three groups of spermatozoa in teleost fishes, according to their degree of evolution. The most primitive spermatozoon type is found in carp and pike, more elaborate spermatozoa are present in trout, while the

guppy's male gametes are the most complex in structure. The general tendency is an elongation in the head region and a fusion of mitochondria to form a collar-like structure around the posterior part of the head.

Mattei (1969) observed that several organelles of the spermatozoon migrate during spermiogenesis and he devised a classification system for spermatozoa of teleosts mainly based upon these movements. He distinguishes four consecutuve phases in the maturation process of the spermatid : 1) spermatid formation; 2) migration of the centrioles; 3) rotation of the nucleus; 4) migration of the mitochondria. According to this system, two types of teleost fishes exist, one in which spermiogenesis completes the four steps, the other in which the nucleus does not rotate.

In depth studies of spermiogenesis are necessary to classify the spermatozoa of channel catfish in either of the two systems, which was beyond the scope of this study.

Unlike teleost fishes, those of the class Chondrichthyes, have close morphological relationships, and are also related spermatologically. In teleosts the enormous diversity in spermatological aspects and species specificity exists which could be useful for taxonomic purposes.

No apparent differences in ultrastructural morphology existed between the wild and domestic stock of channel catfish, neither between the age groups, nor the three consecutive areas of the spermatogenic part of the testes.

Gonadal Sperm Concentration

The mean overall gonadal sperm concentration was approximately 5.5×10^9 spermatozoa/g wet testicular tissue (Tables 5 and 18), and fluctuated during the late fall and winter, but increased from February on (Tables 6 and 20).

Some teleost species show the greatest spermatogenic activity soon after the spawning season (Lofts, 1968). During the winter testicular activity is suspended or reduced but resumes as the spawning season approaches. The degree of this post nuptial spermatogenesis varies widely among species and can be influenced by environmental factors. For the channel catfish, however, age seemed to be the factor influencing sperm concentration most, with 3-year-old fish producing more spermatozoa per gram of wet testicular tissue than the 2-year-olds (Tables 6 and 20). Since the older fish were also the larger ones, some of the effect might have been associated with size. The correlation coefficient between body weight and overall gonadal sperm concentration was 0.39 and highly significant ($P < 0.01$) (Appendix table 7).

When mean gonadal sperm concentration among the three areas of the spermatogenetic part of the testes were compared (Table 19) little variation was observed. Production seemed to be equivalent throughout the testes. Mean values fluctuated without any apparent trend during the late fall and winter, but spermatogenetic production increased steadily

Table 18. Overall mean, standard error of the mean, and coefficient of variation of gonadal sperm concentration of 35 male channel catfish, all months (December 1971-April 1972), stocks, ages, and testes areas combined

Variable	Number of observations	Mean	Standard error	Coefficient of variation
Gonadal sperm concentration ($x10^9$ spermatozoa/g of wet testicular tissue)	102	5.5879	0.1345	24.31

Table 19. Mean, standard deviation, and range of gonadal sperm concentration in the three testes areas, of 39 male channel catfish, all months (November 1971-April 1972), stocks, and ages combined

Variable	Number of observations	Mean	Standard deviation	Range
		($x10^9$ spermatozoa/g wet testicular tissue)		
Gonadal sperm concentration				
Anterior area	39	5.7343	2.2571	1.5998-9.7038
Medial area	39	5.7078	2.7401	0.8466-11.4516
Posterior area	39	5.5681	3.1004	1.4262-11.0042

Table 20. Mean and standard error of the mean of gonadal sperm concentration of 34 male channel catfish, grouped by month, by stock, by age, and by testes area

Number of observ.	Month	Stock	Age	Testes area	Gonadal sperm concentration[a]
18	Dec.'71	Both	Both	All	4.4614±0.9681
18	Jan.'72	Both	Both	All	6.2847±0.9681
21	Feb.'72	Both	Both	All	4.1002±0.8963
21	March'72	Both	Both	All	5.1753±0.8963
24	April'72	Both	Both	All	7.5729±0.8384
48	All	D.A.[b]	Both	All	5.4542±0.5928
54	All	Yazoo[c]	Both	All	5.7067±0.5589
48	All	Both	2[d]	All	4.1633±0.5928
54	All	Both	3[e]	All	6.8542±0.5589
34	All	Both	Both	Ant.	5.6192±0.2330
34	All	Both	Both	Med.	5.7314±0.2330
34	All	Both	Both	Post.	5.4131±0.2330

a $\times 10^9$ spermatozoa/g of wet testicular tissue.

b Wild fish from Lake des Allemands, Louisiana.

c Domestic fish from Yazoo City, Mississippi.

d Fish in their second year.

e Fish in their third year.

beginning in February. Both stocks had similar gonadal sperm concentration (Table 20).

The slight discrepansies between values of mean gonadal sperm concentration between Tables 5 and 18 and Tables 6 and 20 respectively, are due to the fact that in Tables 5 and 6, data collected during November 1971 were included and the overall gonadal sperm concentration was calculated from the weighed averages obtained for the three consecutive areas of the testes.

The values obtained for gonadal sperm concentration must be regarded as conservative estimates. During the procedure, some spermatozoa were probably released from the testicular tissue, by handling of the material.

Traditionally channel catfish males with wide heads, a secondary sexual characteristic, are preferred as brood-stock. The correlation coefficient between the width of the head and the gonadal sperm concentration was 0.51 (Appendix table 7). Although this correlation was highly significant ($P < 0.01$), it would be presumptuous to conclude that a biological cause-effect relationship between the two variables existed.

Scant information is available on gonadal sperm production in fishes. Clemens and Grant (1965) studied spermatozoal concentration in seminal fluid of common carp and rainbow trout, after injections of pituitary extracts. Sperm counts varied between 24.7×10^6 and 38.7×10^6/ cc semen in carp, and between 20.3×10^6 and 26.4×10^6/cc in trout.

Billard et al. (1971) used a technique similar to the one in this study, to determine the gonadal sperm concentration in rainbow trout males. He calculated an average production of 57.8×10^9 spermatozoa/g testicular tissue/year. Spermatozoal concentrations ranged between 6×10^9 and 25×10^9 cells/cc ejaculate. Collection of sperm every 2 days gave the highest total number of spermatozoa recovered, which only represented 22% of the gonadal sperm reserves. A sharp decline in gonadal sperm concentration of rainbow trout occurred during the 2 months following spawning activities. By the 4th month, all spermatozoa in the testes were resorbed.

In channel catfish, however, sperm production seemed to fluctuate during the fall and winter without a trend, but increased during the early spring, as the spawning season approached. This fact may be of vital importance in experimental research attempting year around spawning. If a suitable technique of collecting milt from male channel catfish could be developed, and egg formation stimulated by optimal environmental conditions and hormonal injection, artificial fertilization and cryogenic preservation of male gametes could be fully explored.

Salinity and Temperature Influence on Spermatozoal Motility in Vitro

The four spermatozoal samples tested had the same initial motility of 4, before the experiment started.

The osmotic pressures of the 0, 2, 5, 8, and 11°/oo saline solutions were respectively 8, 71, 152, 230, and 318 milliosmoles. The osmotic pressure of the blood plasma averaged 344 milliosmoles for the four fish. In a preliminary test it was shown that no motility occurred in 11°/oo, therefore this salinity was eliminated for the test. The pH of the solutions was 7.1, 7.5, 7.4, and 7.2 for respectively 0, 2, 5, and 8°/oo.

Salinity had more influence on depression of spermatozoal motility than temperature. In 0°/oo all spermatozoa were immotile after 5 minutes, regardless of the temperature and are therefore not represented in the figures. In Figures 6, 7, 8, 9, and 10 the horizontal time scale was expanded within the 0 to 5 minutes range, in order to be able to represent the mean motility score after elapse of 1 minute. In all experiments, the mean motility score reached 0 after 720 minutes, except at 24 C in salinity 5°/oo (Figure 8). Some mean motility values overlapped in the 360 to 720 minutes range, and since superimposing lines would obscure the figure, the motility score was drawn for one of the temperature or salinity levels only in that part of the figure (Figures 6 and 9).

The three temperatures tested had a similar influence on motility (Figure 6) and no significant ($P > 0.05$) interaction with salinity (Appendix table 8). Figure 7

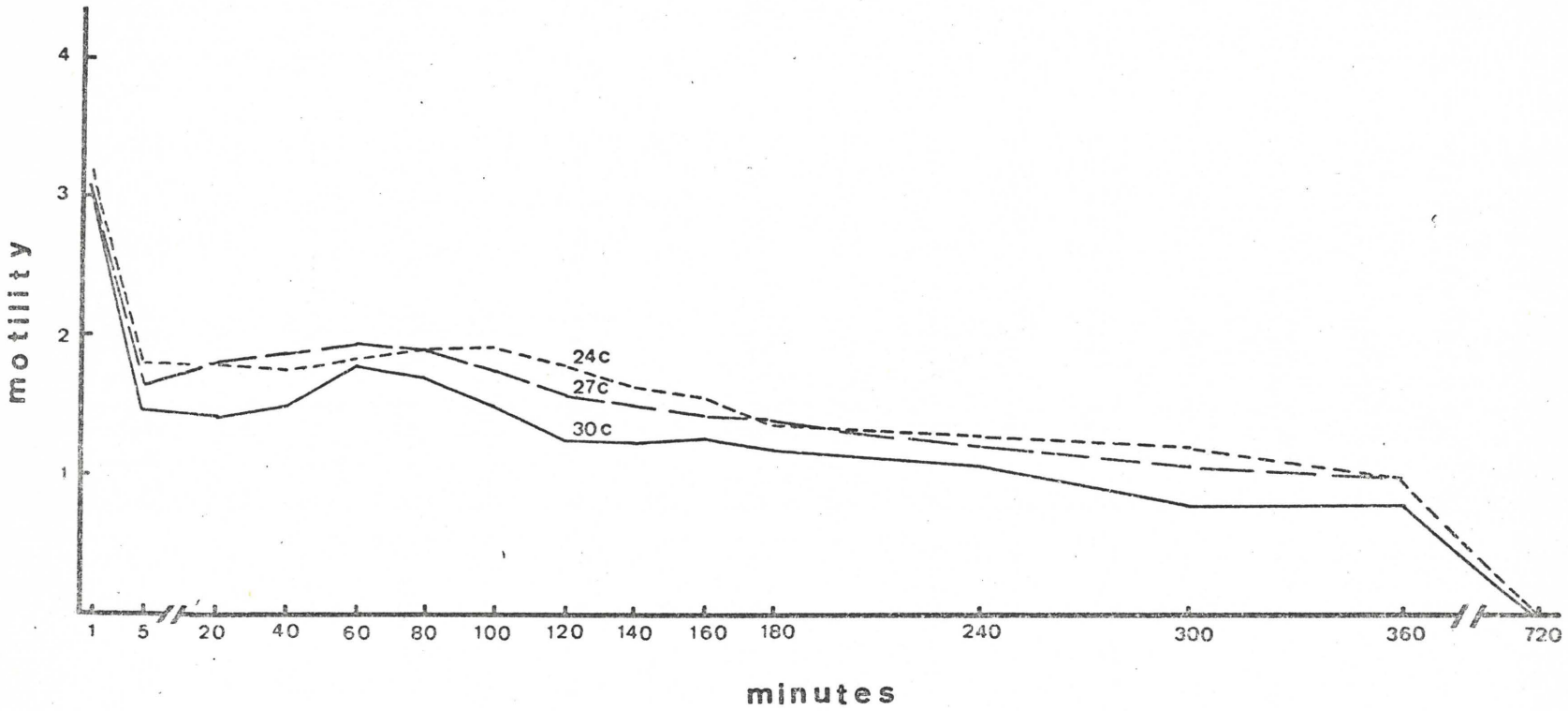

Figure 6. Mean motility score of channel catfish spermatozoa at three temperatures in function of time, all tested salinities combined.

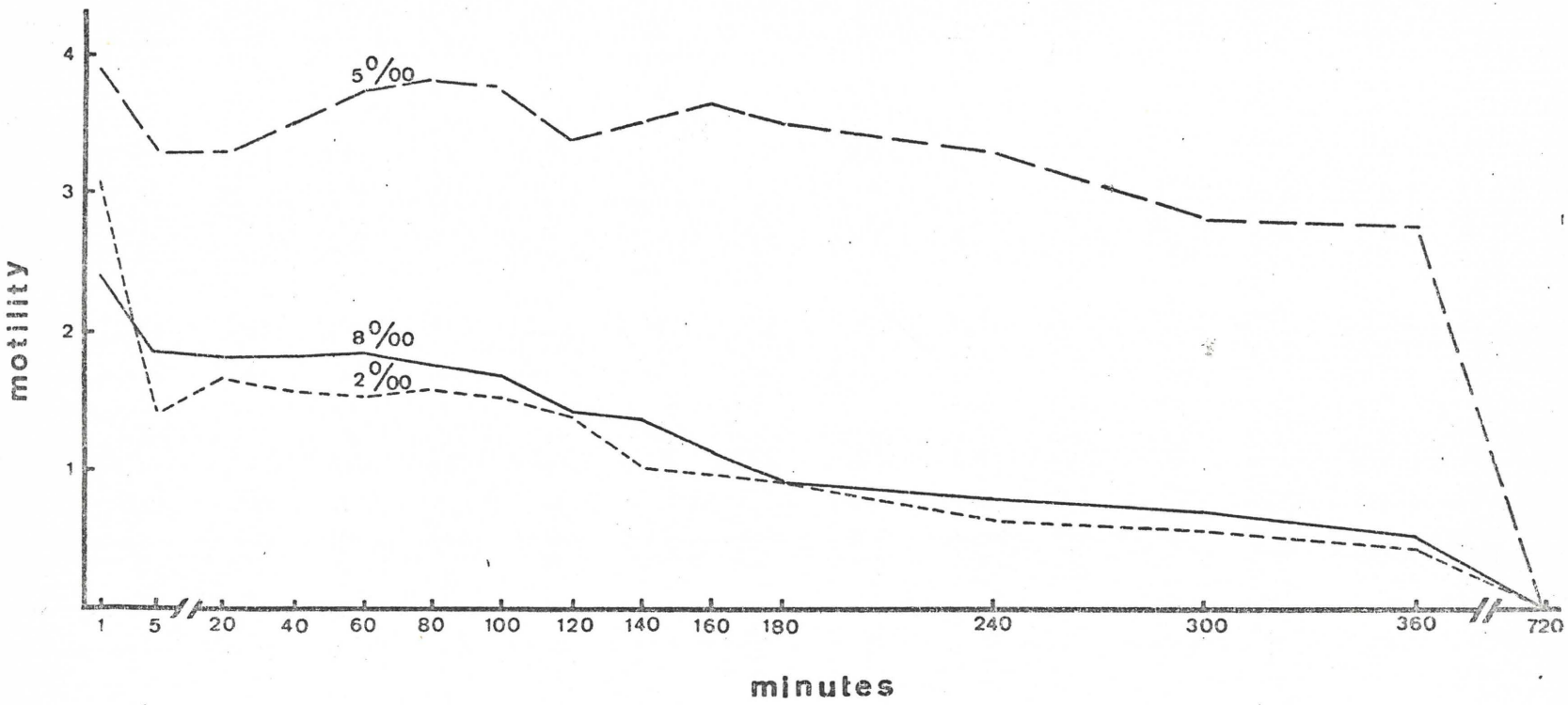

Figure 7. Mean motility score of channel catfish spermatozoa suspended in three salinities in function of time, all tested temperatures combined.

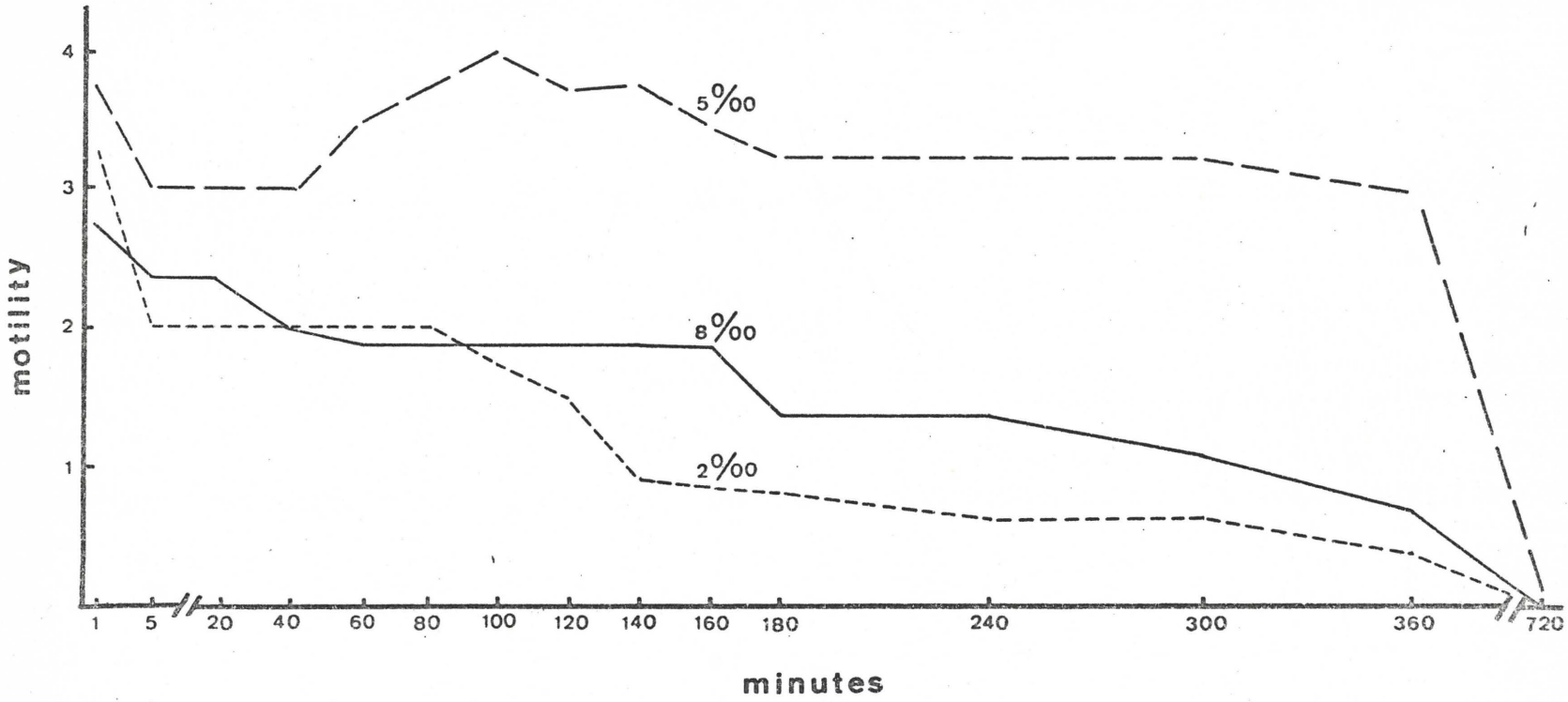

Figure 8. Mean motility score of channel catfish spermatozoa suspended in three salinities at 24 C, in function of time.

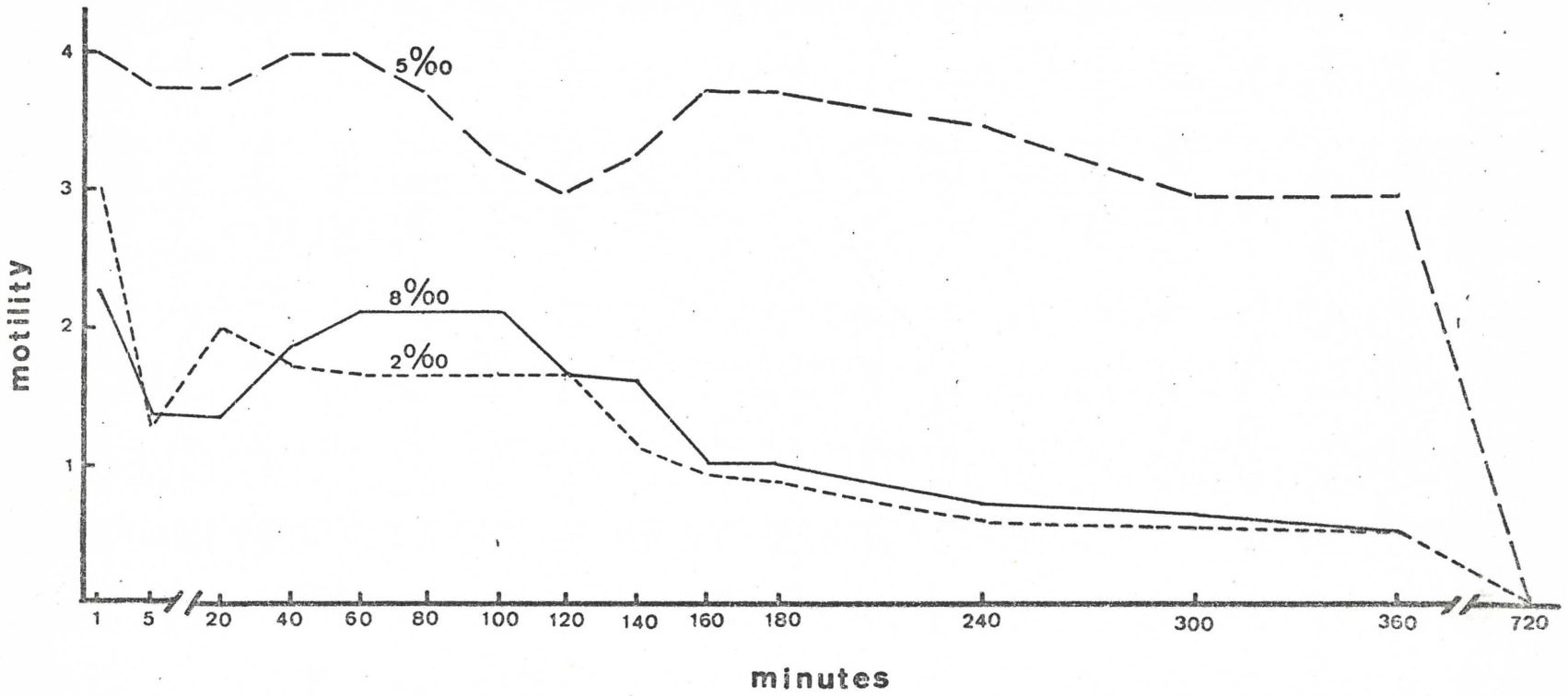

Figure 9. Mean motility score of channel catfish spermatozoa suspended in three salinities at 27 C, in function of time.

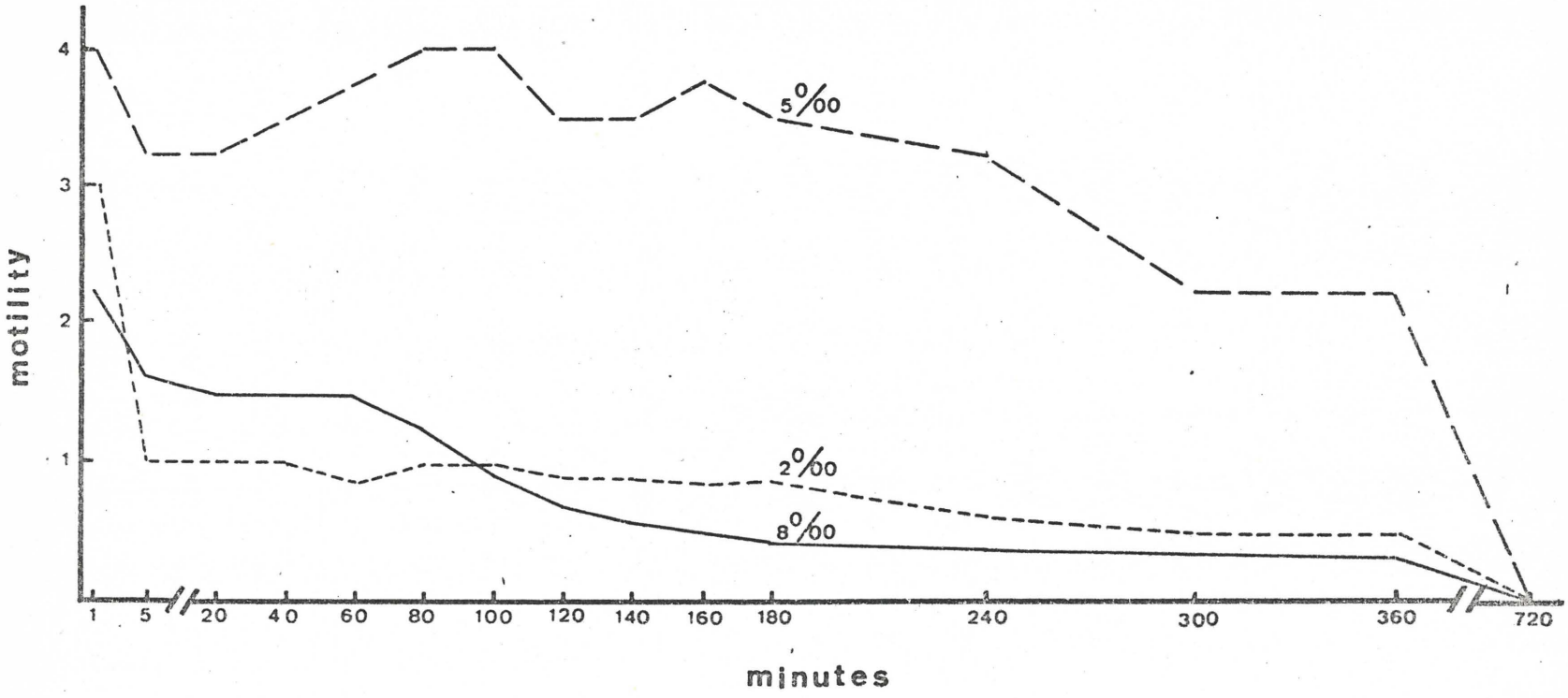

Figure 10. Mean motility score of channel catfish spermatozoa suspended in three salinities at 30 C, in function of time.

shows that motility depression was more severe in 2 and 8°/oo than in 5°/oo, which was optimal in all tests. The mean motility score was highest at this salinity (Table 21) and sustained vigorous spermatozoal movements longest at the three temperatures tested (Figures 8, 9, and 10).

Some minor variations in motility depression occurred within each salinity, at the three tested temperatures, but the overall response remained the same for each salinity level (Figures 8, 9, and 10).

In all cases depression was very severe during the first 5 minutes, but in some instances motility either increased within 15 minutes or remained constant for 15 minutes and then increased (Figures 8, 9, and 10). A second depression of the motility occurred after some time, followed in some cases by a second minor increase in activity, and then decreasing .

After 12 hours all motility had ceased except at 5°/oo and 24 C, where an occasional spermatozoa continued to move.

Physical, chemical and biological factors can influence spermatozoal motility, usually through mediation of biochemical processes on a molecular level, where a change in one of the environmental conditions may have many consequences in cell metabolism (Mann, 1954). Among the factors that usually affect spermatozoal motility most are pH, electrolytes, and temperature (Mann, 1954).

Spermatozoa of rainbow trout are only motile within

Table 21. Mean motility score of four spermatozoal samples grouped by fish, by temperature, by salinity, and by time, and overall mean, all groups combined

Number observ.	Fish	Temp. (C)	Salinity (°/oo)	Time (minutes)	Motility score
180	1	All	All	Complete	1.52
180	2	All	All	Complete	1.61
180	3	All	All	Complete	1.27
180	4	All	All	Complete	1.60
240	All	24	All	Complete	1.62
240	All	27	All	Complete	1.56
240	All	30	All	Complete	1.32
180	All	All	0	Complete	0.20
180	All	All	2	Complete	1.21
180	All	All	5	Complete	3.23
180	All	All	8	Complete	1.36
48	All	All	All	1	3.10
48	All	All	All	5	1.66
48	All	All	All	20	1.71
48	All	All	All	40	1.73
48	All	All	All	60	1.78
48	All	All	All	80	1.80
48	All	All	All	100	1.73
48	All	All	All	120	1.56
48	All	All	All	140	1.47
48	All	All	All	160	1.42
48	All	All	All	180	1.32
48	All	All	All	240	1.20
48	All	All	All	300	1.03
48	All	All	All	360	0.94
48	All	All	All	720	0.10
720	All	All	All	Complete	1.50

a pH range of 7.8 to 8.0 (Schlenk, 1933), while those of longjaw mudsucker, Gillichthys mirabilis Cooper, were not affected between pH 4.8 and 10.15 (Weisel, 1948). The pH among the salinities used in this study varied little and probably affected the spermatozoal motility only to a minor extend if any, but its individual influence was not determined.

The osmotic pressure of the surrounding medium affects the spermatozoal morphology and metabolism. Seminal plasma of Atlantic salmon averaged 232 milliosmoles (Hwang and Idler, 1969) while Horton et al. (1967) reported a sucrose solution of 272 milliosmoles to be optimal for maintaining normal spermatozoal morphology.

In this study, motility was sustained best at 152 milliosmoles and depressed drastically at 230 milliosmoles. Channel catfish could not be forced to ejaculate and the osmolality of the seminal fluid could not be determined, but when compared to blood plasma, the optimal osmolality was hypotonic.

Spermatozoal motility of sturgeon, Acipenser sp. was studied in salinities ranging from 0 to 8°/$_{oo}$ (Drabkina, 1961). The duration of progressive movement was highest in 2°/$_{oo}$ but averaged only 6.6 minutes, while some motion was also sustained until 16 minutes. In salinities of 6 and 8°/$_{oo}$, however, motility was instanteneously depressed.

Spermatozoal resposes to outside stimuli seem to differ drastically from one fish species to another.

Doroshev and Gorelov (1964) investigated the influence of various salinities on two chalcalburnus species, Chalcalburnus chalcoides danubicus Antipa and Ch. chalcoides aralensis Berg, and on common carp. Chalcalburnus sperm remained motile in salinities of 3 to 7.5 °/₀₀ for 30 to 50 minutes, compared to 2 to 3 minutes in fresh water. Carp sperm remained motile for 7 to 8 minutes in fresh water but 30 to 50 minutes in salinities ranging from 3 to 7.5°/₀₀. Sperm activity reduced at 9 to 10°/₀₀ and ceased almost instantaneously at higher salt concentrations for the three species.

Compared to these results, channel catfish spermatozoa remain motile much longer. At 360 minutes after introduction into the saline solution, motility in 2, 5, and 8°/₀₀ was observed, with the highest value in 5°/₀₀. Motility score was not checked in the following 6 hours, but since activity in 5°/₀₀ was still very vigorous it may be assumed that motion continued for at least 2 to 3 hours.

In the literature none of the authors mentioned if the sperm used was obtained directly from the testicular tissue or from an ejaculate. Therefore comparison is difficult, because spermatozoal motility might be affected differently in either situation.

Lindroth (1947) suggested that the length of the swimming time of several fresh water fish spermatozoa decreased exponentially with increasing water temperature. Comparison with this study is difficult because Lindroth (1947) used temperatures below 24 C and experimented mainly with cold water species.

Because of the enormous diversity in experimental procedures, the complexity of the interactions among stimuli and the array of responses by spermatozoa of different fish species, it would be presumptuous to make any conclusive statement concerning the influence of environmental factors on spermatozoal motility in fishes.

SUMMARY AND CONCLUSIONS

1. Males of a wild stock of channel catfish, Ictalurus punctatus (Rafinesque) from Lake des Allemands, Louisiana were compared with males belonging to a domestic stock from Yazoo City, Mississippi for several parameters relating to reproductive physiology.
2. During the study (November 1971 - April 1972) 24 male fish of each stock were examined, consisting of 12 2-year-olds and 12 3-year-olds.
3. The data were analysed by least square analyses of variance and corrected for unequal numbers. The means were calculated within and among each source of variation (month, stock, age, testes part, or testes area) and the range recorded. The correlation coefficient among 35 variables was computed.
4. Secondary sexual characteristics were usually more pronounced in the larger fish
5. The mean body weight of all fish examined was 687 g with a range of 142 to 2,050 g. When stocks were compared within an age group, the domestic fish were considerably larger, while in each stock the 3-year-olds outgrew the 2-year-olds.

6. The testes consisted of a white broadly lobular spermatogenic part and a pinkish featherly glandular part. The spermatogenic part weighed on the average 1.14 g, the glandular one only 0.56 g. A significant increase in both parts was noticed starting in March and continuing during April. In six small 2-year-old fish belonging to both stocks, no testes could be recognized although the male secondary sexual characteristics existed.
7. In each stock a case of azoospermia was encountered. Both 3-year-old fish showed all male secondary sexual characteristics and had well developed testes but which lacked spermatozoa.
8. The spermatogenic part of the testes was devided in three consecutive areas and the variables measured in each one separately. For most properties, however, these three areas were very similar.
9. Spermatozoal motility was generally high with most cells in progressive motion and some vibrating in loco.
10. The percent live spermatozoa in the testes was determined with an eosin-nigrosin differential stain. An overall average of 73.2% of the spermatozoa teased from the testes were alive.
12. Spermatozoal morphology was investigated using the light and electron microscope. Sperm dimensions,

aberrations, and ultrastructures were investigated. The channel catfish spermatozoon has a rounded head 2.3 um long and 2.4 um wide, a collar-like midpiece containing diffuse mitochondria and 1.6 µm in length and 3.1 µm in width, and a 94.9µm long flagellum.

13. Biflagellar spermatozoa each arising from an individual centriolar complex were present in all examined fish at an average of 4.7%. An apparently split flagellum singly implanted in the head but separated longitudinally in some areas was recognized in 9.4% of the spermatozoa.

14. The gonosomatic index averaged only 0.22% and reached its highest value in April with 0.32%.

15. The gonadal sperm concentration averaged 5.5×10^9 spermatozoa/g wet testicular tissue and reached a maximum in April with 7.4×10^9. Both stocks produced a similar number of spermatozoa per gram of wet testicular material. As a group the 3-year-old fish contained in the testes twice as many spermatozoa per gram tissue as the 2-year-olds. A highly significant ($P < 0.01$) correlation coefficient of 0.51 existed between the width of the head and gonadal sperm concentration.

16. In a separate experiment the influence of 0, 2, 5, and 8°/₀₀ salinity at 24, 27, and 30 C on spermatozoal motility in vitro was tested during 720 minutes at

20 minute intervals.

17. The saline solution of $5^{o}/_{oo}$ proved to be the optimal one among those tested at the three temperature levels. Vigorous spermatozoal motility was maintained for at least 360 minutes in this salinity. At $0^{o}/_{oo}$ motility was completely depressed within 5 minutes for all temperatures tested. Temperature seemed to have less impact on spermatozoal motility than salinity. One fish of each age group within each stock was examined but the spermatozoa of these four fish responded in the same manner to the applied environmental stimuli.

18. For most variables no apparent differences between the wild and the domestic stock could be discerned. Variation in some of the variables was probably associated with size or age and a few such as gonadal sperm concentration with the season.

19. It is hoped that some aspects of this study will be continued during the spawning season and early fall to obtain comparable data on the male reproductive cycle of channel catfish expanding over a year.

20. It is suggested that future research will give attention to inducing ejaculation and ovulation, artificial fertilization of eggs and cryogenic preservation of spermatozoa, which could possibly

lead to year aroung production of fry which would be a tremendous economic advantage and facilitate improvements of stocks through selective breeding.

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APPENDIX

Appendix table 1. Summary of analyses of variance of body and testes measurements, gonosomatic index, and gonadal sperm concentration of 40 male channel catfish (November 1971 - April 1972)

Sources of variation	Degrees of freedom	Mean squares and significance								
		Body		Head		Testes			Gonosomat. index	Gonadal sperm concentration
		Weight	Total length	Width	Circumference	Weight	Length	Volume		
Month	5	65060.4506	1974.7345	231.03845	1485.3322	2.9464^{+a}	1179.7430	2.4678^{+}	0.0310^{++}	9435x10^{-12}
Stock	1	1717441.6873^{++}	110951.9680^{++}	3360.0747^{++}	16091.8841^{++}	12.6048^{++}	11740.7368^{++}	14.0963^{++}	0.0065	11920x10^{-12}
Age	1	3123665.8378^{++}	141953.3708^{++}	7222.8937^{++}	48452.5357^{++}	32.9293^{++}	34099.5182^{++}	33.0028^{++}	0.1000^{++}	723226x10^{-12++}
Stock x age	1	168979.5957	430.0725	22.2395	696.0973	1.7653	444.9824	1.9749	0.0006	9710x10^{-12}
Error	31	99322.9902	1876.0092	128.5128	1953.2371	1.0098	507.0007	0.9742	0.0079	43000x10^{-12}

[a] + Significant statistical difference at the $P < 0.05$ level.

[b] ++ Highly significant statistical difference at the $P < 0.01$ level.

Appendix table 2. Summary of analyses of variance of percent live spermatozoa and motility score of 35 male channel catfish (December 1971-April 1972)

Sources of variation	Degrees of freedom	Mean squares and significance	
		% Live spermatozoa	Motility score
Month	4	309.9216	7.7730++[b]
Stock	1	28.6017	0.9600
Age	1	178.2150	0.1067
Month x stock	4	120.3482	3.3424+
Month x age	4	364.8444	1.1457
Stock x age	1	224.4817	6.0000+
Month x stock x age	4	266.6807	2.1845
Error A	15	491.1806	0.8444
Area	2	159.450+[a]	0.0067
Month x area	8	38.7325	0.0578
Stock x area	2	86.4867	0.0200
Age x area	2	10.2200	0.2467
Month x stock x area	8	56.0568	0.0887
Month x age x area	8	47.9139	0.0289
Stock x age x area	2	46.5867	0.0200
Month x stock x age x area	8	30.9340	0.0554
Error B	30	37.2639	0.1444

[a] + Significant statistical difference at the $P < 0.05$ level.

[b] ++ Highly significant statistical difference at the $P < 0.01$ level.

Appendix table 3. Summary of analyse of variance of spermatogenic and glandular parts of the testes, of 35 male channel catfish (December 1971-April 1972)

Sources of variation	Degrees of freedom	Mean squares and significance			
		Weight	Length	Volume	Width
Month	4	1.2553	675.8411+[a]	1.2438	112.9594
Stock	1	7.0760++[b]	5198.41++	6.8069++	262.44+
Age	1	16.2030++	17187.21++	15.7371++	2097.64++
Month x stock	4	0.8201	189.5976	0.8532	67.7758
Month x age	4	0.6421	240.9517	0.5976	43.7824
Stock x age	1	0.4662	30.2500	0.4212	49.0000
Month x stock x age	4	0.5863	441.6468	0.5706	18.3365
Error A	15	0.4804	172.6833	0.4558	54.3333
Part	1	5.8803++	17239.6900++	5.9585++	408.0400++
Mont x part	4	0.0958	328.7386+	0.0914	2.2873
Stock x part	1	0.0861	4.41	0.0894	17.6400
Age x part	1	1.4467+	630.01+	1.4424	67.2400
Month x stock x part	4	0.2312	318.8804+	0.2092	16.7627
Month x age x part	4	0.1189	181.2214	0.1074	6.5135
Stock x age x part	1	0.2810	600.2500+	0.2237	1.9600
Month x stock x age x part	4	0.1696	53.6608	0.1624	10.23
Error B	15	0.1816	95.2167	0.1792	17.2667

[a] + Significant statistical difference at the $P < 0.05$ level.

[b] ++ Highly significant statistical difference at the $P < 0.01$ level.

Appendix table 4. Summary of analyses of variance of spermatozoan dimensions of 35 male channel catfish (December 1971 - April 1972)

Sources of variation	Degrees of freedom	Mean squares and significance					
		Total length head & midpiece	Length head	Length midpiece	Width head	Width midpiece	Length flagellum
Month	4	0.1399[+a]	0.1634[++b]	0.0808[+]	0.1206[++]	0.0417	106.05764[+]
Stock	1	0.0136	0.0418	0.0077	0.0307	0.0418	170.1224[+]
Age	1	0.0136	0.0077	0.0008	0.0307	0.0691	54.4053
Month x stock	4	0.0558	0.0129	0.0579	0.0153	0.0562	18.2062
Month x age	4	0.0225	0.0424	0.0205	0.0238	0.0256	63.3217
Stock x age	1	0.2184[+]	0.1032	0.0008	0.0000	0.0213	34.3042
Month x stock x age	4	0.0048	0.0477	0.0539	0.0638	0.0617	35.2122
Error A	15	0.0313	0.0270	0.0242	0.0171	0.0327	30.6073
Area	2	0.0538	0.0213	0.0077	0.0179	0.1254	8.6773
Month x area	8	0.0793	0.0549	0.0360	0.0388	0.0488	6.9159
Stock x area	2	0.1544	0.0418	0.0538	0.0179	0.0264	19.1260
Age x area	2	0.0111	0.0230	0.0162	0.0077	0.0691	18.9506
Month x stock x area	8	0.0974	0.0895	0.0650	0.0262	0.0696	26.5815
Month x age x area	8	0.0239	0.0235	0.0375	0.0380	0.0372	7.4376
Stock x age x area	2	0.0264	0.0367	0.0111	0.0179	0.0111	28.4828
Month x stock x age x area	8	0.0417	0.0425	0.0129	0.0041	0.0235	12.4173
Error B	450	0.0890	0.0581	0.0406	0.0378	0.0642	36.6294

[a]+ Significant statistical difference at the $P < 0.05$ level.

[b]++ Highly significant statistical difference at the $P < 0.01$ level.

Appendix table 5. Summary of analyses of variance of percent normal spermatozoa and percent spermatozoa with morphological aberrations of 35 male channel catfish (December 1971-April 1972)

Sources of variation	Degrees of freedom	Mean squares and significance		
		% Normal spermatozoa	% Spermatozoa biflagellar	% Spermatozoa with split flagellum
Month	4	1766.2569++[b]	86.9168	1127.9519++
Stock	1	513.3750+[a]	72.1067	198.3750++
Age	1	284.2817	0.4267	265.3350+
Month x stock	4	445.5516+	19.6504	402.0960++
Month x age	4	346.8806+	14.9152	245.1921+
Stock x age	1	2.2817	18.0267	7.0417
Month x stock x age	4	109.3668	17.1488	78.5619
Error A	15	113.1083	45.0278	53.4306
Area	2	22.0217	2.1950	12.9017
Month x area	8	9.4494	0.3260	7.6067
Stock x area	2	16.3950	2.3617	9.5550
Age x area	2	4.7217	1.8317	5.8550
Month x stock x area	8	12.8771	1.600	7.0022
Month x age x area	8	15.1258	0.5580	13.3010
Stock x age x area	2	12.7617	1.5517	10.9017
Month x stock x age x area	8	9.2165	3.8130	8.9053
Error B	30	33.8417	3.1611	18.8639

[a] + Significant statistical difference at the $P < 0.05$ level.

[b] ++ Highly significant statistical difference at the $P < 0.01$ level.

Appendix table 6. Summary of analysis of variance of gonadal sperm concentration of 34 male channel catfish (December 1971-April 1972)

Sources of variation	Degrees of freedom	Mean squares and significance
		Gonadal sperm concentration
Month	4	40.2152
Stock	1	6.7275
Age	1	65.4001
Month x stock	4	9.3834
Month x age	4	2.5986
Stock x age	1	0.9533
Month x stock x age	3	1.4778
Error A	15	16.8701
Area	2	0.9027
Month x area	8	2.058
Stock x area	2	0.6451
Age x area	2	1.1137
Month x stock x area	8	0.2845
Month x age x area	8	0.6316
Stock x age x area	2	0.3957
Month x stock x age x area	6	0.6300
Error B	30	1.8460

Abbreviations used in Appendix table 7

BWt	Body weight
BL	Body length
WiH	Width head
CiH	Circumference head
TeWt	Testes weight
TeL	Testes length
TeV	Testes volume
GSI	Gonosomatic index
GSC	Gonadal sperm concentration
WtS	Weight spermatogenic part of testes
LS	Length spermatogenic part of testes
VS	Volume spermatogenic part of testes
WiS	Width spermatogenic part of testes
WtG	Weight glandular part of testes
LG	Length glandular part of testes
VG	Volume glandular part of testes
WiG	Width glandular part of testes
LA	Percent live spermatozoa in anterior area of testes
LM	Percent live spermatozoa in medial area of testes
LP	Percent live spermatozoa in posterior area of testes
MA	Motility score of spermatozoa in anterior area of testes
MM	Motility score of spermatozoa in medial area of testes
MP	Motility score of spermatozoa in posterior area of testes
NA	Percent normal spermatozoa in anterior area of testes
NM	Percent normal spermatozoa in medial area of testes
NP	Percent normal spermatozoa in posterior area of testes
2FA	Percent biflagellar spermatozoa in anterior area of testes
2FM	Percent biflagellar spermatozoa in medial area of testes
2FP	Percent biflagellar spermatozoa in posterior area of testes
SFA	Percent spermatozoa with split flagellum in anterior area of testes
SFM	Percent spermatozoa with split flagellum in medial area of testes
SFP	Percent spermatozoa with split flagellum in posterior area of testes
GSCA	Gonadal sperm concentration in anterior area of testes
GSCM	Gonadal sperm concentration in medial area of testes
GSCP	Gonadal sperm concentration in posterior area of testes

Appendix table 7. Correlation coefficients in percent, and statistical significance, between 35 variables relating to the reproductive physiology of 40 male channel catfish (November 1971-April 1972)

+ Statistically significant at the $P < 0.05$ level.

++ Statistically highly significant at the $P < 0.01$ level.

	BWt	BL	WIH	CiH	TeWt	TeL	TeV	GSI	GSC	WtS	LS	VS	WiS	WtG	LG	VG	WiG	LA	LM	LP	MA	MM	MP	NA	NM	NP	2FA	2FM	2PP	SFA	SPM	SPP	GSCA	GSCM	GSCP
BWt																																			
BL	91++																																		
WIH	92++	94++																																	
CiH	84++	84++	86++																																
TeWt	89++	82++	88++	80++																															
TeL	83++	91++	92++	83++	84++																														
TeV	89++	84++	85++	81++	99++	88++																													
GSI	30	40++	50++	45++	63++	57++	62++																												
GSC	39++	49++	61++	54+	38++	45++	35+	35+																											
WtS	85++	80++	88++	77++	96++	84++	95++	69++	35+																										
LS	75++	84++	86++	74++	77++	94++	80++	60++	45++	82++																									
VS	85++	85++	88++	79++	96++	86++	95++	68++	34+	100++	83++																								
WiS	75++	79++	83++	69++	80++	84++	86++	61++	51++	87++	89++	87++																							
WtG	85++	73++	75++	71++	92++	72++	91++	48++	38++	77++	68++	77++	59+																						
LG	79++	82++	33++	86++	79++	90++	81++	44++	40++	72++	70++	73++	63++	77++																					
VG	81++	76++	75++	72++	89++	78++	92++	46++	32+	74++	65++	75++	60++	96++	81++																				
WiG	71++	67++	79++	65++	77++	68++	74++	50++	51++	72++	65++	71++	78++	72++	62++	67++																			
LA	- 8	-13	- 2	-14	- 7	-17	-14	-17	4	-13	-21	-15	-10	2	- 8	-11	7																		
LM	-11	-14	- 3	1	- 1	- 9	- 5	- 1	- 8	- 6	-12	- 7	- 4	5	- 2	- 3	13	81++																	
LP	-12	-16	-11	-11	- 6	-15	- 9	-15	0	-13	-20	-14	- 8	6	- 4	- 1	10	84++	86++																
MA	- 4	- 5	- 4	-13	- 2	0	- 1	- 9	- 2	- 2	- 5	- 2	5	- 3	8	1	-13	- 1	- 1	2															
MM	- 9	- 7	- 9	-16	-11	- 6	- 9	-13	- 2	-10	-13	-10	- 2	-10	6	- 5	-18	- 8	- 6	- 4	85++														
MP	- 3	- 2	- 2	- 8	1	6	3	- 2	0	1	0	1	10	1	12	5	- 6	- 6	- 4	0	90++	86++													
NA	31+	31+	37+	26	37+	41++	37+	29	5	35+	43++	37+	38+	34+	32+	34+	51+	16	22	8	- 5	-16	-11											
NM	37+	31+	57+	25	35+	40++	36+	27	20	54++	46++	54+	32+	33+	26	52+	30	15	23	11	-15	-21	-26	74++										
NP	31+	30+	36+	28	35+	39++	36+	24	3	33+	42++	34+	37+	33+	30	35+	29	23	30+	19	-19	-23	-23	86++	90++									
2FA	-37+	-58++	-46++	-33+	-38++	-48++	-40++	-30	-11	-42++	-49++	-42++	-44++	-28	-32	-30+	-14	8	- 8	8	- 4	- 5	2	-71++	-58++	-56++								
2PM	-31+	-34+	-31+	-25	-30	-36+	-31+	-23	-22	-32+	-42++	-32+	-37+	-23	-21	-26	-10	10	- 5	5	- 8	-12	4	-43++	-66++	-46++	80++							
2PP	-22	-27	-24	-18	-26	-31+	-28	-24	- 4	-27	-30+	-27	-32+	-22	-20	-24	- 8	- 4	-22	- 5	- 1	-17	- 5	-60++	-71++	-65++	82++	89++						
SFA	-25	-25	-31+	-20	-32+	-34+	-31+	-25	- 3	-28	-35+	-29	-31+	-32+	-28	-30	-33+	-22	-25	-12	7	22	13	-97++	-70++	-85++	51++	24	46++					
SPM	-26	-28	-32+	-20	-31+	-34+	-30+	-24	-16	-28	-38+	-28	-29	-30	-22	-29	-33+	-23	-26	-16	22	31+	30+	-73++	-94++	-91++	37+	38+	49++	76++				
SPP	-28	-26	-34+	-22	-33+	-36+	-33+	-20	- 2	-30	-37+	-31+	-27	-32+	-28	-31+	-32+	-27	-28	-20	27	33+	29	-81++	-82++	-96++	38+	23	42++	86++	91++			
GSCA	29	43++	42++	24	22	35+	21	15	87++	21	36+	20	41++	20	30	20	35+	11	- 3	7	- 2	4	0	2	12	- 2	- 6	-13	2	0	-10	2		
GSCM	38+	42++	49++	31+	40++	36+	35+	34+	93++	33+	35+	32+	42++	45++	33+	34+	48++	16	5	9	- 4	- 7	- 6	21	36+	18	-17	-31+	-15	-20	-31	-17	73++	
GSCP	31+	41++	42++	28	33+	40++	31	31+	95++	30	38+	30	45++	32+	37+	28	45++	- 4	-13	- 3	0	- 1	2	7	24	5	- 6	-22	- 2	- 6	-20	- 6	74++	84++	

Appendix table 8. Summary of analysis of variance of motility score of spermatozoa of four male channel catfish

Sources of variation	Degrees of freedom	Mean squares and significance
		Motility score
Fish	3	4.4775
Temperature	2	6.2089+[a]
Salinity	3	287.4449++[b]
Temperature x salinity	6	2.1972
Error A	33	1.7779
Time	14	19.9078++
Temperature x time	28	0.1240
Salinity x time	42	3.0522++
Temperature x salinity x time	84	0.3032++
Error B	504	0.1200

[a] + Significant statistical difference at the $P < 0.05$ level.

[b] ++ Highly significant statistical difference at the $P < 0.01$ level.

VITA

Edmonde Jeanne Micheline Jaspers (Mony) was born in Antwerp, Belgium on April 3, 1940. She attended elementary and secondary school in Antwerp and graduated from the Royal Lyceum for Girls in June 1959.

She was then employed full-time by the Ministery of National Education and Culture in Brussels.

In October 1962, she enrolled at the State Faculty of Agricultural Sciences in Ghent, Belgium and graduated with the diploma of Agricultural engineer, Section Waters and Forests in June 1967.

The same year, she was awarded a Fulbright-Hays Travel Grant and was admitted to the Graduate School of Louisiana State University, Baton Rouge, in September 1967. She was granted a graduate research assistantship in the School of Forestry and Wildlife Management, Fisheries Section, and obtained the degree of Master of Science in Fisheries in August 1969.

In September 1969, she enrolled in the Department of Marine Sciences of Louisiana State University, Baton Rouge, and continued working as a graduate research assistant in the Fisheries Section.

Presently she is a candidate for the degree of Doctor of Philosophy with a major in Marine Sciences and a minor in Fisheries.